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Objects (*Const., Art. II*): . . . "(a) To promote broadly the advancement of active research in all branches of dental science and in related phases of the sciences that contribute directly to the development of dentistry, and which add to the knowledge of the mouth and teeth and of their relations to the body as a whole; . . . (b) to encourage and facilitate coöperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology; (c) to the end, particularly, that dentistry may render cumulatively more perfect service to humanity." (The Association has been using "dentistry" and "stomatology" as synonyms.)

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2. (To a book)—Doe, J. A., *The Dental Practice* (Chicago, Smith: 1930), p. 114.

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Dreizen, Mann, Cline and Spies (p. 213) found a definite difference in the buffer capacity of saliva in caries resistant and caries susceptible individuals. They also made observations on the relationship of the nutritional state and caries susceptibility tests.

Becks and Wainwright (pp. 267, and 275) give three analyses of saliva content data. These represent statistical studies of their data and add to our increasing basic knowledge of saliva.

Hemmens, Blayney, Bradel and Harrison (p. 195) carefully studied the "cultivable microbic flora" of dental plaques from the time of tooth eruption until after caries appeared. Several forms decreased in number with caries progress but lactobacilli definitely increased.

Bibby, Zander, McKelleget and Labunsky (p. 207) add more material toward solution of the fluorine-caries problem. They used fluorides in a prophylactic mixture and in a mouth wash, unfortunately not in comparable groups.

Karshan, Tenenbaum, Karlan, and Leonard continue an investigation of the blood factors and B.M.R. of individuals with periodontal disease. They find a few significant positive relationships and several important negative correlations (p. 247).

Slanetz and Brown (p. 223) tested the glycerite of hydrogen peroxide against oral microorganisms. Their results justify their projected clinical trial of the drug.

Berg, Burrill and Fosdick (p. 231) found that the rate of putrefaction of saliva is greater from patients with periodontal disease. This is part of a larger program on the chemistry of periodontal disease.

Two contributions on pathologic lesions of the mouth appear in this issue, one from the Army Medical Museum and one from Texas. Bernier and Thompson report on myoblastoma (p. 253) and Wellensiek on eosinophilic granuloma. This reminds us that a new group has been pledged to advancement of oral pathology research and teaching. The Registry of Oral and Dental Pathology at the Army Institute of Pathology has sponsored an *American Academy of Oral Pathology*. Charter fellows and provisional officers have been selected. Fellowship will be conferred only on qualification by training and examination to future fellows but membership is on a more liberal basis.

The International Association for Dental Research celebrated its 25th Anniversary with a banquet in New York. Dr. Krasnow gives a report of the proceedings on page 293.

H. B. G. R.

THE MICROBIC FLORA OF THE DENTAL PLAQUE IN RELATION TO THE BEGINNING OF CARIES[1]

ELIZABETH S. HEMMENS, J. R. BLAYNEY, S. F. BRADEL, AND R. W. HARRISON

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The bacterial flora of the oral cavity is one of the most complex to be found in or on the human body. This may be attributed in part, at least, to the variety of environments which may be found in the mouth and to the constant inflow of microorganisms from the air and food. This rich field of investigation has intrigued many workers since the early work of W. D. Miller (1) and a very diverse literature has accumulated. Numerous contributions have dealt with the microorganisms indigenous to the mouth and with changes in the flora related to specific diseased conditions. Most of the reports have been restricted to some part of the flora, usually to a single genus or species. Among those studies having a less specialized viewpoint and making notable contributions to knowledge in this field should be included the work of Kligler on the relationship of bacteria to dental caries (2), of Bloomfield (3) and of Brailovsky-Lounkevitch (4) on the evolution of the oral flora in infants, of Howitt and Fleming on changes in the flora associated with different diets (5) and of Bibby (6) and of Brooke (7) on smears from different areas in the mouth.

Little attention has been given by workers in either dental or bacteriological fields to the microbic flora of the dental plaque, in spite of the frequently repeated observation, first made by G. V. Black, that in the earlier stages of the carious process the lesion is always found beneath a plaque composed of bacteria and other microorganisms. These masses are most often found on tooth surfaces which are protected from the normal cleansing mechanisms of the oral cavity and in areas difficult to reach with ordinary dental instruments. The paucity of information in this field of dental research is also partly attributable to the numerous technical difficulties of sampling and cultivation of the small amounts of material available.

It seems apparent, however, that a search for the bacterial agent or agents which may be responsible for the earliest stages of the carious process must be made among those microorganisms in and under the plaque. If decay is initiated by local bacterial action the responsible microorganism should be found in plaques covering carious enamel and, conversely, if the relationship is a direct one, should be absent when the surface is found to be caries inactive. Indirect evidence of differences in bacterial flora between caries active and caries free tooth surfaces is found in the work of Stephan (8). He has shown that the pH

[1] Received for publication April 15, 1946.

of carious lesions is lower, in general, than that of sound tooth surfaces and that, after the mouth is rinsed with sugar solution, the plaques on caries active surfaces attain a lower pH and maintain increased acidity over a longer period than do plaques from sound surfaces.

More direct evidence of qualitative differences in the bacterial flora of caries active and inactive tooth surfaces is offered by observations reported from this laboratory (9). Bacteria resembling groups I and II of Hadley, Bunting and Delves (10) were found in a high percentage of stained smears of plaques from carious areas and were not found in $\frac{3}{4}$ of plaques from non-carious teeth. These results were confirmed by a further investigation in which smear examinations and cultures on acid media were made from the same plaques. These experiments, while they demonstrated an association of lactobacilli with the carious lesion in most instances, showed enough lack of correlation that another approach was indicated. It is obvious that activity leading to caries must be present for some time before decalcification of the enamel can be detected by roentgenological or clinical examination. In any large group of plaques collected from clinically sound enamel there are likely to be some from areas in a "pre-clinical" state of caries activity. Such plaques should harbor the postulated bacterial agent causing decay and failure to consider the data in this light would hinder correct interpretation of the results. On the other hand, it is possible that the agent initiating caries does not remain throughout the evolution of one lesion and that other microorganisms may be involved in later stages in the process. Thus, some plaques from more advanced lesions might not contain the bacteria in question.

In order to overcome these difficulites to some extent and obtain a clearer picture of the role of all of the bacterial flora associated with dental decay, a study has been made of cultures from a series of plaques from selected interproximal tooth surfaces, the first plaques in the series being obtained before contacts were established and successive ones being removed at intervals throughout the period of evolution of the enamel lesions.

METHODS

Patients in whom the premolar teeth were erupting were selected from among those being seen at the clinic. Plaques were removed with especially designed, fine, dental scalers from proximal surfaces of 1 or more teeth and were individually prepared for culture according to methods described previously (11). A careful clinical examination, with explorer and mirror was made of the area and bite-wing radiographs were taken. Radiographs were taken subsequently at intervals of 3 months and the remainder of this procedure was repeated every 6 weeks for each site studied until lesions had developed and had been present for at least 6 weeks. Most of the areas were studied for 6 months after appearance of the lesion.

Cultures of the plaques were made on blood agar (pH 7.2), acid tomato agar (pH 5.0)(12) and in acid glucose broth (pH 5.0)(13) and were incubated anaerobically in an atmosphere of 10% CO_2 at 37°C for 4 days. Differentiation of the

various types of bacteria which grew was made from stained smears of agar colonies or broth cultures. Subcultures were prepared, when needed, for further study and identification. Except where otherwise noted, the nomenclature of Bergey's Manual of Determinative Bacteriology (14) has been used.

Only areas which were shown by clinical and x-ray examination to have remained free from recognizable caries for at least 6 months after the initial examination were included in the study.

The clinical record of each area was studied after positive diagnosis of caries had been definitely confirmed and the time when the first sign of carious activity had been detected either by roentgenological or clinical methods, was established as the reference point for that lesion. The culture data from all cases were assembled so that the observations coincided at the reference points and, therefore, at each of the preceding and succeeding examination periods. Using this method it was possible to compare and to combine for purposes of analysis the results of examinations at various intervals preceding and following the first appearance of decay. The relative frequency of occurrence of each microorganism was determined by dividing the total number of areas examined at a given examination interval into the number of these areas from which the microorganism was obtained. The resulting fractions were converted into numbers per 100 surfaces in order to provide for direct comparison of percentage incidence of different organisms as well as for convenience in plotting the changes in incidence of each organism. Thus, a series of figures representing frequency of isolation was obtained for each microorganism recovered, over a period of time during which a carious lesion developed on the tooth surface being studied.

RESULTS

Plaque culture studies were initiated on a total of 269 tooth areas of 85 children. Some of the children were dropped from the study because of failure to attend the clinic regularly. Several of the areas originally chosen were dropped because there was clinical evidence of caries by the time of the second or third examination period and the period of precarious observation was considered inadequate for the purposes of the study. After these were eliminated there remained 87 areas on the teeth of 44 children which became recognizably carious within 6 months to 2 years after the first examination. A total of 939 plaques were removed for cultures from these areas, 611 of them before there was clinical or x-ray evidence of caries and 328 of them after lesions had been detected. The average length of observation was 1½ years but some areas were studied as long as four years.

In Table I are listed all the microorganisms that were found in 2% or more of the plaques. Differentiation was made as precise as possible on the basis of morphological and colonial characteristics. In many cases these observations placed organisms within a genus but further study usually was found to be necessary for species identification. Accordingly, collections have been made of representative cultures and final identification of species has been deferred for further study on these selected strains. It is apparent, however, that the plaques contained at least 27 varieties of microorganisms.

There were several species of *Corynebacterium* represented in the plaque cultures. In a representative collection of 11 strains studied for differential

TABLE I

Incidence of Some Oral Microorganisms in Plaque Cultures in Relation to the Beginning of Caries

ORGANISM	PERIODS OF OBSERVATION* PLAQUE CULTURES YIELDING SPECIFIED MICROORGANISMS						DIFFERENCE IN INCIDENCE BETWEEN CARIOUS AND PRECARIOUS PERIODS
	Precarious (211 plaques)		Transitional (400 plaques)		Carious (328 plaques)		
	#	%	#	%	#	%	
α streptococci (smooth colony)	155	73	253	63	173	53	−20%
Leptotrichia	102	48	175	44	109	33	−15%
Fusiformis nucleatus	81	38	131	33	78	24	−14%
Gram positive rod, plump	51	24	74	18.5	37	11	−13%
Neisseria (yellow colony)	75	35	107	27	76	23	−12%
Actinomyces	94	44	146	37	109	33	−11%
α streptococci (rough colony)	86	41	145	36	102	31	−10%
γ streptococci (smooth colony)	75	35	125	31	88	27	−8%
γ streptococci (ridged colony)	50	24	100	25	57	17	−7%
Other *Fusiformis* spp.	52	25	97	24	62	19	−6%
Micrococci (rough colony)	19	9	27	7	16	5	−4%
Neisseria (gray colony)	64	30	114	29	90	27	−3%
Micrococci (tetrads)	8	4	9	2	5	1.5	−2.5%
Gram negative filaments	18	8.5	58	14.5	24	7	−1.5%
Gram positive diplococcobacilli	20	9	14	3.5	26	8	−1%
Gram positive rods (small)	68	32	128	32	101	31	−1%
Gram negative rods	60	28	113	28	92	28	0
Veillonella (yellow colony)	41	19	94	23.5	63	19	0
Yeasts	13	6	21	5	19	6	0
Staphylococcus aureus	15	7	25	6	28	9	+2%
Staphylococcus albus	36	17	92	23	62	19	+2%
Veillonella (gray colony)	45	21	106	27	79	24	+3%
γ streptococci (rough colony)	36	17	98	24.5	71	22	+5%
Aciduric streptococci†	169	80	353	88	282	86	+6%
Diphtheroids	140	66	314	78	241	74	+8%
β hemoloytic streptococci	25	12	70	17.5	73	22	+10%
Lactobacilli	28	13	122	30	154	47	+34%

* The carious period included cultures made at the time of the first appearance of caries and up to 117 weeks later; the transitional period included the 36 weeks immediately preceeding the time of first detection of caries and the precarious period included cultures made up to 96 weeks prior to the transitional period.

† The aciduric streptococci, i.e., those streptococci isolated on acid media, include alpha, beta and gamma forms. All of many cultures which were tested grew also on media at pH 7.2. The group overlaps other groups shown in the table.

characterization, 2 resembled *C. hoffmanni*, 2 were either *C. acnes* or *C. hoagii*, 1 was *C. flavidum* and 1 appeared to be like *C. diphtheriae* but no virulence tests were made. The other 4 strains could not be classified in any of the known species on the basis of physiological characteristics. The classification of the

non-pathogenic members of this genus needs to be more satisfactorily worked out, especially in relation to the oral types.

At least 3 species of *Neisseria* were found in a group of 18 subcultures. Four strains of "yellow colony" and 4 of "gray colony" *Neisseria* were found to be *N. sicca*. A smooth "gray colony" strain was *N. subflava*. Two rough "gray colony" strains were *N. crassus*[2]. Three "gray colony" and 1 "yellow colony" strains conformed to descriptions of *N. perflava*. Three "yellow colony" strains grew poorly without fermenting any of the carbohydrates tested and may have have been *N. catarrhalis*.

Results of a separate study of the alpha streptococci of the mouth will be reported in a later paper but preliminary findings indicate that there is as great a degree of heterogeneity among these strains as has been reported for collections from other sources. Only a few strains could be called *Streptococcus salivarius* on the basis of characteristic growth on 5% sucrose agar as described by Niven, Smiley and Sherman (16) when the strains were tested soon after isolation. A number of strains resembled *Str. lactis* and some showed the characteristics of *Str. fecalis*. The majority of strains in the collection probably fall within the group designed by Niven, Smiley and Sherman (16) as *Str. mitis*.

The group of aciduric streptococci which were found in almost all of the acid glucose broth cultures appeared to be made up of strains of alpha, gamma and, rarely, beta hemolytic streptococci similar to those isolated from blood agar cultures of the same specimens. It is evident that many strains of the streptococci of the mouth are able to grow at pH 5.0 and that this property was not especially characteristic of any one group. Among the streptococci of plaque origin which grew in acid glucose broth there were no types from carious areas which did not appear also in plaque cultures from sound enamel.

No attempt has been made, as yet, to classify the obligate anaerobes except morphologically. The basis for designating one type of fusiform bacilli as *Fusiformis nucleatus* has been given in a previous paper (17). In this same paper, the characteristics of the genus *Veillonella* were compared with those of certain similar forms isolated from the mouth and it was concluded that most of the strains studied were *V. gazogenes* var. *syzygios*. The types described in the present series are undoubtedly of this genus but species differentiation has not been made.

Further work on classification of the more important members of the oral flora both from plaques and from saliva is being carried on and will be described in a later communication.

The data on incidence of various microorganisms in plaque cultures have been condensed into 3 divisions: (1) precarious, the entire period of observation prior to 36 weeks before the first evidence of carious activity was noted; (2) transitional, the period of 36 weeks immediately preceding the earliest evidence evidence of carious lesion; and (3) carious, the period following demonstration of a lesion. The designation of a period as "transitional" was made primarily

[2] This is a lactose fermenting *Neisseria* which is not listed in Bergey's Manual but is described in Topley and Wilson's textbook (15).

for convenience in considering the data. However, it is evident that some carious activity must have preceded the time at which a lesion was first detected. The absence of a detectable lesion on examination 6 weeks before the time of its first demonstration does not rule out the possiblity of subclinical activity at that time. Similarly, one cannot deny, on the basis of information available at present, the possibility of carious activity at earlier periods of observation. It has frequently happened that the evidence of caries activity from direct clinical examination was questionable 6 weeks or more after the lesion had been definitely demonstrated roentgenographically and many of the lesions have progressed at a very slow rate after having been detected both by x-ray and explorer. It is probable, therefore, that the initial stages of the process occur many weeks in advance of the first observable damage to the enamel and it seems justifiable to label as "transitional" a period of time well in advance of the date of first detection of the lesion. A period of 36 weeks was chosen arbitrarily. It has been designated as a transitional period rather than a period of initial, preclinical or inapparent caries because of the lack of information through which its limits might be fixed.

The frequency of isolation of each of the 27 kinds of microorganisms in cultures made in the precarious, transitional and carious periods is shown in Table I. Assuming that those forms which produce the carious lesion would be found with greater frequency when the lesion has appeared and that those microbic types which have no part in the carious process would either not change in incidence or might be found less frequently, the table has been so arranged that these relationships will be shown. The order of listing the microorganisms is by the magnitude of the difference between the incidence in the carious period and the incidence in the precarious period. Thus, those bacteria at the top of the table decreased in frequency of isolation with the appearance of the lesion and those lowest in the list increased in incidence.

A majority of the microorganisms recovered showed some degree of negative correlation with the carious process. The alpha type streptococci (*Str. viridans*), gram positive filamentous rods (*Leptotrichia*, *Actinomyces* species) and anaerobic fusiform bacilli (*F. nucleatus* and others) decreased most markedly in incidence with the onset of the lesion. *Neisseria*, some gamma streptococci and an unidentified plump gram positive rod were also less common when a lesion was present. Some of the micrococci, *Veillonella*, gram negative filamentous rods and unidentified gram positive diplococcobacilli and small rods occurred in small numbers and showed little change in incidence. A group of gram negative rods, probably largely of the enteric group, were found in the same relative numbers in all 3 periods.

Several of the bacterial types were found somewhat more frequently in the transitional period than in the precarious or carious periods. Most of these, *Veillonella*, *Staphylococcus albus*, one type of gamma streptococcus and gram negative filaments occurred in relatively small numbers and probably are not important in connection with the process of decay. Diphtheroids and aciduric streptococci were the bacteria most commonly isolated from the plaque cultures and both were somewhat more frequently found during the transitional period.

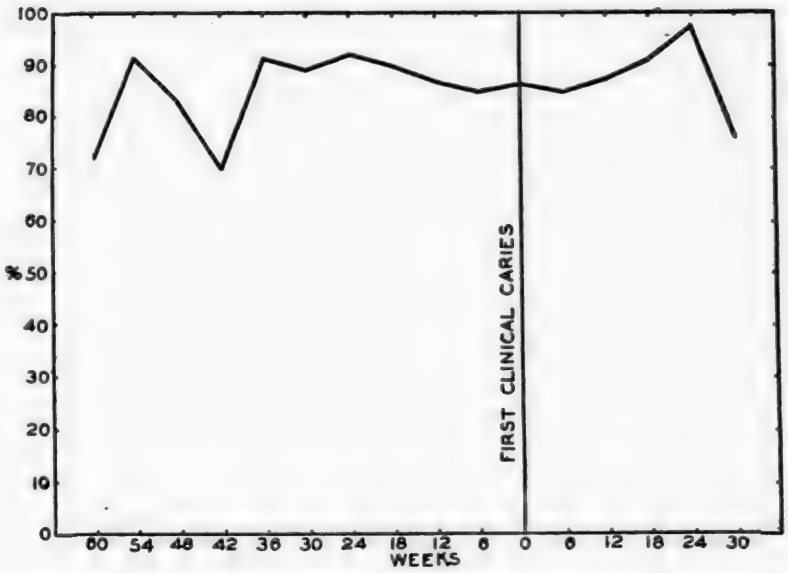

Fig. 1. Percentage incidence of aciduric streptococci

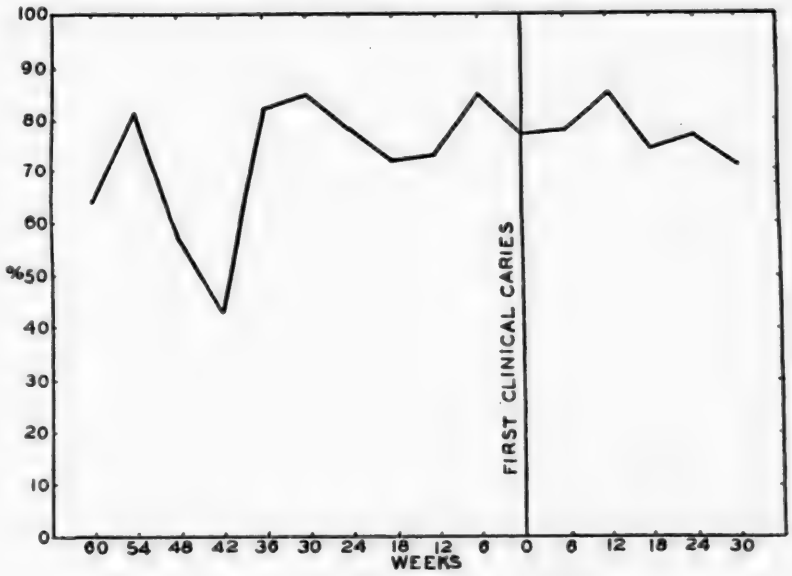

Fig. 2. Percentage incidence of diphtheroids

However, line graphs of the incidence of these 2 groups (*figs. 1* and *2*), show no progressive increase during the time when the initial stages of decay must be occurring as would be expected if these bacteria were active in producing the lesion.

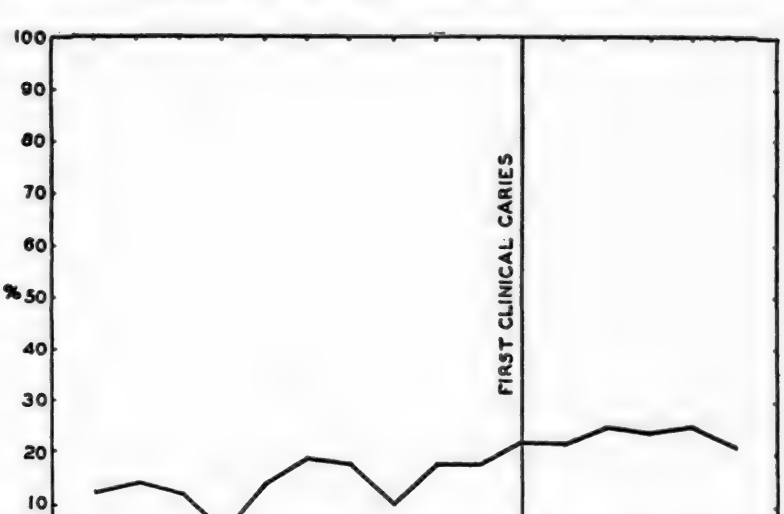

Fig. 3. Percentage incidence of beta hemolytic streptococci

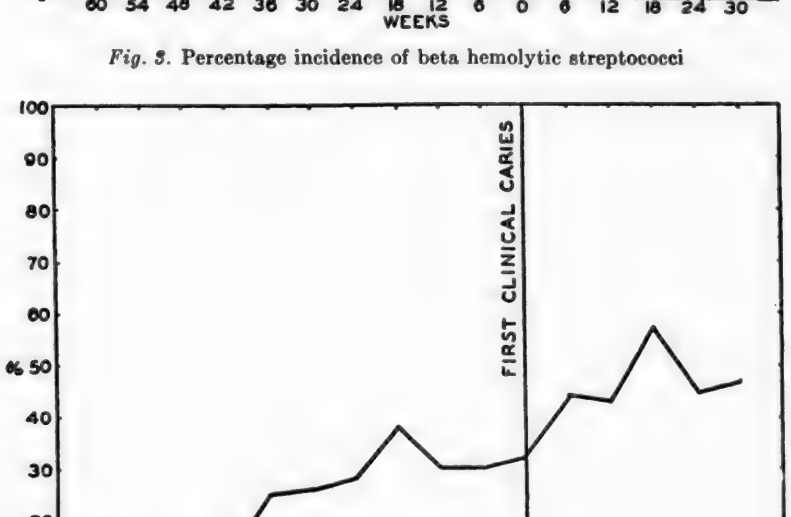

Fig. 4. Percentage incidence of lactobacilli

The 2 organisms which showed a definite and progressive increase in incidence associated with the onset of the lesion were *Str. hemolyticus* and lactobacilli (*figs. 3* and *4*). These streptococci were relatively uncommon, however, even at

the point of greatest incidence and there is little evidence from this study that they have any role in the production of caries. With the possibility in mind that these organisms might produce a lesion in the absence of other agents, a separate analysis of the data obtained from 13 areas from which lactobacilli were rarely or never isolated was made. No microorganism, including the beta hemolytic streptococci, showed any more positive correlation with the progress of caries than was observed in the complete series.

The most marked change in incidence observed in the study occurred with the lactobacilli. The incidence curve for these organisms rose steadily, as shown in *fig. 4*, from the 48th week preceeding clinical caries to the 18th week after caries was demonstrated, i.e., until the lesion had become established. These bacteria appear to be associated with development of the lesion to a greater degree than any other type which was found in the cultures.

DISCUSSION

The problem of the etiology of dental decay is one of the most perplexing in dental research. The theory of Miller, explaining caries on the basis of dissolution of the tooth enamel by acids of bacterial origin, has gained support from many investigators, partly because of its plausibility and partly as the result of slowly accumulating evidence in its favor. Final proof, however, is still to be obtained. The complexity of the oral flora and the meagreness of knowledge about this bacterial population under normal conditions make investigations hard to plan and results difficult to interpret.

Most discussions of this problem have centered on a single active agent but the possibility of several agents or of an association of bacteria being responsible for dental decay, as suggested by Bibby (18), must be considered. Many of the oral flora, for example, the fusiform bacteria, are able to produce enough acid in the test tube to decalcify teeth but very little is known about what these forms would do in their natural environment. Study of the symbiotic and synergistic relations of the oral flora might yield useful information, but much more detailed study on the bacterial flora of the tooth surface is needed before it will be possible to determine the nature and degree of association of various bacterial forms with the caries process.

In a study of the relation of the bacterial flora to caries, the plaque is the logical source of culture material because of its association with the enamel surface in areas which are prone to decay.

It has been possible to make satisfactory cultures from the plaque in spite of various technical difficulties which arise mainly from the minuteness of the specimen. The tenacious nature of the plaque, however, facilitates its removal from the tooth surface and makes it possible to free it from gross superficial contamination from saliva and mucous membranes.

The primary purpose of this investigation has been to ascertain what microorganisms are contained in the cultivable indigenous flora of the tooth surface before and after caries appears. An attempt has been made to obtain this information by culturing plaques obtained many months previous to the appear-

ance of lesions as well as plaques from surfaces on which caries activity was occurring.

It is not possible to set a limit on the time necessary to produce a carious lesion. Although the evidence presented in this paper indicates that the process is usually slow and insidious, it may start in some individuals as soon as a plaque containing the causative organisms is formed and contact with the adjacent tooth has become established.

If it were possible definitely to fix the beginning of caries with the appearance of a single species of microorganism in the plaque, or with the appearance of one or more of a small number of species, more accurate information could be obtained concerning the speed or rate at which the initial carious changes take place. This could be done, perhaps, with some degree of assurance if the causative organism or organisms were known.

Assuming that the etiological agent is included among those microorganisms that were cultivated from the plaque, the results of the experiments here reported indicate either that the techniques of isolation are very inadequate or that the responsible organisms are sporadically present. The maximum incidence found for any microorganisms in the carious period was only 86 per 100 plaques, this being the group of aciduric streptococci, and the incidence of the various components of this group ranged downward from 53 per 100 to 17 per 100. The alpha streptococci, which were found in slightly more than half the plaques from carious surfaces, were encountered much more often (73%) in the plaques from noncarious surfaces. The lactobacilli, which definitely increased in incidence throughout the period in which the lesions developed, were not isolated from more than 57% of the plaques of any one time interval.

Filamentous forms, actinomyces and leptotrichia, invariably were seen in smears but frequently failed to grow in cultures of the plaques. This observation is in keeping with previous findings from this laboratory and elsewhere.

The authors believe, however, that the culture methods were adequate for isolation of the more readily cultivable forms. It is suggested, therefore, that these forms either were not continuously present in the plaques or were frequently present in such small numbers, actual as well as relative, as to make isolation difficult or impossible. In either case, caries-producing activity of these organisms would be intermittent or fluctuating in character.

The situation is further confused by probable wide fluctuations in other factors, for example, the availability of fermentable substrate. It seems apparent that the initial stages of dental caries may proceed sporadically and by imperceptible degrees, and it seems reasonable to conclude that the process is usually extended over a considerable period of time.

There did not appear to be any modification of the flora of the plaque due to repeated removal for cultivation since it was found many times that plaques were renewed within 48 hours. In one experiment, removal of plaques from the same surface every 48 or 72 hours over a period of at least a month did not affect the cultivable flora to any recognizable degree. It seems unlikely, therefore, that the changes observed could be modifications in the flora enforced by removal of the plaque every 6 weeks.

SUMMARY

The cultivable microbic flora of dental plaques has been studied beginning with the newly erupted tooth and continuing through the period of early enamel decay until after carious lesions had become well established.

Changes in the frequency of isolation in culture of several types of bacteria have been observed. Leptotrichia and actinomyces, alpha hemolytic streptococci, fusiform bacteria and several species of *Neisseria* were among those forms which decreased in incidence with the progress of the lesion. Lactobacilli were the only bacteria for which there was definite evidence of increased incidence in association with the development of carious lesions.

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PRELIMINARY REPORTS ON THE EFFECT ON DENTAL CARIES OF THE USE OF SODIUM FLUORIDE IN A PROPHYLACTIC CLEANING MIXTURE AND IN A MOUTHWASH[1]

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Previous reports (1, 2) have shown that the activity of dental caries can be reduced by bringing aqueous solutions of sodium fluoride into contact with the freshly cleaned tooth surfaces of children. Unfortunately, the methods so far employed for this purpose are somewhat time-consuming and require the employment of techniques not usually used by dentists or dental hygienists. Therefore it seemed desirable to determine whether alternative and simpler methods of topical treatments with fluoride solutions might not bring about comparable reductions in caries activity. For this reason it was decided to study the effects of: 1. Incorporating sodium fluoride in a cleaning mixture which could be used in a routine way by dental hygienists giving oral prophylaxes, and 2. The use of a mouthwash containing sodium fluoride.

Because it had been shown (3) that fluoride solutions buffered to an acidity of pH 4 reduce the solubility of enamel and dentin much more than do ordinary aqueous fluoride solutions, it seemed advantageous to use such acidulated preparations in these studies.

I. PROPHYLAXIS STUDIES

Two separate studies were made to test the effect on caries of giving prophylaxes with fluoride-containing cleaning mixtures. One was carried out on the school children in Millville, Mass. and the other on girls attending the school at St. Vincent's Orphanage in Cambridge, Mass. Both studies followed the same lines. The children presenting in the clinics were examined with mirror and explorer on the first visit, and all fillings, cavities or questionable caries recorded on anatomical charts. Facilities for x-ray examination were not available. The right side of the mouth was then cleaned by the dental hygienist in the ordinary way with a rubber cup or sometimes an engine brush using a paste made by mixing pumice and hydrogen peroxide. Thereafter the left side of each mouth was cleaned in a like way by using a pumice-peroxide-fluoride mixture. This mixture was prepared by adding one third volume of a 4% solution of sodium fluoride to commercial hydrogen peroxide and adding enough of this liquid to the pumice to make a fluid paste. Because the commercial hydrogen peroxide is highly acidulated, these cleaning mixtures had a pH of approximately 4.0. Two prophylaxes of this sort were given during the year in the Millville study and 3

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in the St. Vincent's study. The teeth of both study groups were reexamined at the end of the year and differences in caries activity between control and fluoride treated sides was estimated. In Millville, 95 children, aged 6 to 15 years, were reexamined and in the St. Vincent's study 47 girls aged 6 to 14 appeared for the second examination. In Millville the children had received no dental treatment for a number of years and most of their mouths were in a deplorable condition A makeshift dental clinic had to be set up using portable equipment and very limited facilities. In St. Vincent's treatments were given in the Orphanage clinic.

TABLE I

Caries increment after 3 prophylaxes—St. Vincents

	SOUND SURFACES AT BEGINNING OF STUDY					
	Total no.	Showing new decay on reexamination		Showing ? decay on reexamination*		
		No.	%	No.	%	
Control side						
Upper teeth: Perm....	588	32	5.4	39	6.1	
Decid....	455	11	2.4	58	11.1	
Lower teeth: Perm....	685	17	2.5	26	3.6	
Decid....	335	8	2.4	26	7.2	
All upper teeth.....	1043	43	4.1	97	8.4	
All lower teeth.....	1020	25	2.5	52	4.5	
All teeth.....	2063	68	3.3	149	6.4	
Fluoride treated side						
Upper teeth: Perm....	610	17	2.8	38	4.8	
Decid....	423	4	0.94	32	7.0	
Lower teeth: Perm....	707	19	2.7	35	4.7	
Decid....	776	8	1.0	49	5.9	
All upper teeth.....	1033	21	2.0	70	6.3	
All lower teeth.....	1483	27	1.8	84	5.4	
All teeth.....	2516	48	1.9	154	5.8	

* In this and subsequent tables "? decay" includes fillings placed in sound surfaces and cavities extending onto sound surfaces.

A comparison between the caries increment in control and fluoride treated sides of the children's mouths is presented in Table I (St. Vincent's) and Table II (Millville). Because, as has been pointed out elsewhere (4), comparison between the effect of fluoride applications on different sides of the same mouths always show more marked differences on the upper teeth than they do on the lower, the findings in these teeth are presented separately.

Comment. The results in both studies indicate that caries activity has been retarded in those teeth which were cleaned with the fluoride containing mixture. In the St. Vincent's study there was approximately 42% less new decay on the treated side than on the control side when all the teeth were included and 51% less if the upper teeth were considered alone. In the Millville study smaller

differences were obatined in the respective zones, the figures being approximately 25% and 31%. The caries reduction produced in the deciduous teeth is striking in the St. Vincent's children, but in the Millville group is, if anything, less marked than in the permanent teeth. The more striking results produced in the St. Vincent's group are probably not primarily related to the fact that 3 treatments were given there as compared with 2 at Millville, but are more likely the result of less favorable working conditions and less cooperative patients, rendering it impossible to give treatments which were equally effective. As a result isolation of the test side of the mouth was less efficient and a greater leakage of the fluoride mixture to the control side of the mouth probably reduced the

TABLE II

Caries increment after 2 prophylaxes—Millville

	SOUND SURFACES AT BEGINNING OF STUDY									
	Total no.	Showing new decay on reexamination				Showing ? decay on reexaminaton				
		No.		%		No.		%		
Control side										
Upper teeth: Perm....	1576	102		6.5		56		3.5		
Decid....	610	84		13.8		12		1.9		
Lower teeth: Perm....	1624	64		3.9		55		3.3		
Decid....	552	52		9.4		12		2.2		
All upper teeth.....	2186	186		8.5		68		3.1		
All lower teeth.....	2176	116		5.3		67		3.0		
All teeth.....	4362		302		6.9		135		3.0	
Fluoride treated side										
Upper teeth: Perm....	1534	66		4.3		47		3.0		
Decid....	615	60		9.7		27		4.4		
Lower teeth: Perm....	1653	54		3.3		49		2.9		
Decid....	531	45		8.5		31		5.8		
All upper teeth.....	2149	126		5.9		74		3.4		
All lower teeth.....	2184	99		4.5		80		3.6		
All teeth.....	4333		225		5.2		154		3.5	

differential between the control and test sides. The finding of smaller differences between the control and treated sides in the lower than in the upper jaws indicates that this was particularly true in respect to the lower teeth.

II. MOUTHWASH STUDY

To determine whether the use of a fluoride containing mouthwash would reduce dental caries volunteers were sought amongst the sophomore students at Tufts College Dental School. The chosen students were examined with a mirror and explorer, in conjunction with bite wing x-ray pictures, and all caries and fillings charted. On the basis of the findings, they were divided into 2 groups each of which showed approximately the same number of patients with high, low and average susceptibility to dental caries. One group was instructed to use,

Mouthwash A and the other Mouthwash B at least 3 times each week. Records were kept of the dates of reissue of mouthwash to the various users. A second control group was made up of students of other classes who were employing no dentifrices or mouthwashes. The content of mouthwash A and B differed only by the inclusion of sodium fluoride in one of them. Which one contained the fluoride was known only to the person compounding them. The mouthwashes were made with a base of sodium acetate-acetic acid buffer at pH 3.2 or 3.7 flavored with saccharine and oil of peppermint and colored with tincture of cudbear. Sodium fluoride was added to the low pH mixture to give a concentration of 1 part in 200. Before use these mouthwashes were diluted in 4 part- of tap water to give a final pH of approximately 4.0 and in one a fluoride concentration of 1 part per 1000. The sodium acetate-acetic acid buffer was used because in laboratory studies fluorides added to this mixture gave maximal reductions of enamel solubility. Unfortunately the flavor of this buffer could not be easily masked and the resultant mouthwash was not well liked by the

TABLE III

Caries increment after use of mouthwashes

	NO. OF PATIENTS REEXAMINED	SOUND SURFACES AT BEGINNING OF STUDY				
		Total sound surfaces	Showing new decay on reexamination		Showing ? decay on reexamination	
			No.	?	No.	?
Fluoride Mouthwash (A)...	31	3349	62	1.8	143	4.3
Control Mouthwash (B)...	15	1701	41	2.4	55	3.2
Control No. Mouthwash...	39	4199	100	2.4	90	2.1

student subjects. This fact together with the absence of close supervisory arrangements resulted in considerable defection amongst the users. A tendency to experiment with one another's mouthwashes also made it necessary to eliminate a number of the subjects from the experiment. At the end of a year all the patients were reexamined and the records of those patients who could not establish faithful use of the mouthwashes were discarded.

A comparison between the occurrence of caries, and questionalbe caries (including fillings, etc.) in the various groups is presented in Table III.

Comment. The findings of this study fail to indicate that the users of the fluoride mouthwash derived any benefit from its use. This result is, as first thought, a little surprising since it is obvious that the teeth in the mouthwash study must have had a considerably greater exposure to the fluoride than did those in either of the prophylaxes experiments reported here on or our Brockton study (1) where the same fluoride concentration was used. There are, however, several reasons why these results should not be interpreted as proving that the activity of dental caries cannot be reduced by the use of fluoride-containing mouthwashes. One of these reasons may be that a clean tooth surface or even frictional contact may be needed to bring about the reaction between the

fluorides and the tooth. A second, and probably more important reason is that there is accumulating evidence to suggest that fluorides are effective in reducing dental decay only when they are brought in contact with teeth which have fairly recently erupted into the mouth. This idea is supported by the results of some clinical tests (1, 5, 6) the field studies of Weaver (7) and some of our laboratory studies (8) which lead us to the conclusion that the originally reactive tooth phosphate becomes progressively less so as continued exposure in the mouth leads to combination with a variety of adventitious and less reactive ions. Obviously then a satisfactory test of the effects of a fluoride mouthwash must be carried out on a larger group of younger people. In line with this conclusion such a study has been instituted.

SUMMARY

A study in which 3 dental prophylaxes using a 1% sodium fluoride-pumice-hydrogen peroxide mixture were given to the teeth on one side of the mouth of 47 children showed an overall reduction in new caries of approximately 43%. A similar study in which 2 fluoride prophylaxes were given to 95 children reduced caries by approximately 25%. It is not believed that the difference in the results is fundamentally the result of the number of treatments given. In both instances caries reduction in the upper teeth was much greater than in the lower teeth. The use, thrice weekly, of an acid (pH 4) mouthwash containing 0.1% sodium fluoride for 1 year did not reduce caries activity in 31 dental students below that of 15 using a control fluoride-free mouthwash, or 39 using no mouthwash.

We are indebted to Dr. Florence Hopkins, former Child Welfare Physician (Dentist) of the Massachusetts Department of Health for providing the opportunity and making arrangements for carrying out the study in Milliville. We also wish to record our appreciation for the assistance given by the Massachusetts Department of Health in making the services of a dental hygienist, Bertha Labunsky, available for this work.

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THE BUFFER CAPACITY OF SALIVA AS A MEASURE OF DENTAL CARIES ACTIVITY[1]

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Repeated observations of patients visiting the Nutrition Clinic of the Hillman Hospital, Birmingham, Alabama, made over a period of 6 years indicate that these people have a remarkably low incidence of dental caries (1). This relative absence of dental caries suggested a number of possible causes, each of which was deemed worthy of study and investigation. The possibilities included(a) relative absence of refined carbohydrates in the dietary,(b) inhibition of the action of the carbohydrate degradation enzymes,(c) exceptionally good oral hygiene and dental care,(d) absence of certain nutritive requirements for the *Lactobacillus acidophilus* in the saliva,(e) effective amounts of fluorine in the water supply, and(f) the relation of caries activity to the buffering capacity of saliva. The result of these studies revealed the following:

a) *Relative absence of refined carbohydrates in the dietary*: These patients are from large families living for the most part in rural Alabama. Their daily diet leaves much to be desired in the light of modern knowledge regarding nutritive needs. Cornbread, biscuits, syrup, and fat pork are the staples, and lean meat and fresh vegetables are eaten only when available, usually in the spring and summer. The total calorific value of their food intake is about 40% below standard. The relative intake of carbohydrates and fats exceeds, however, that of proteins, vitamins, and minerals.

b) *Inhibition of the action of the carbohydrate degradation enzymes*: It has been observed that patients with a vitamin B complex deficiency are also deficient in cozymase and cocarboxylase, which are derived from nicotinic acid and thiamin respectively (2). A lack of cozymase interferes with the carbohydrate degradation process. Cozymase is essential for the Cannizzaro dismutation reaction in which the glucose degradation products, dioxyacetone phosphoric acid and glycericaldehyde phosphoric acid react with each other to from glycericphosphoric acid and phosphoglyceric acid. These products are, in turn, broken down to pyruvic acid and eventually to lactic acid (3). Absence of cocarboxylase interferes with the break down of pyruvic acid to lactic acid. The relative

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We wish to acknowledge the aid rendered by Miss Bessie Carson and Miss Margaret Ward in making the caries activity determinations.

importance of this factor has not as yet been fully ascertained; however, it does appear that the intake of thiamin and nicotinic acid is sufficient to support the growth of the *Lactobacillus acidophilus* in patients with a vitamin B complex deficiency (1).

c) *Exceptionally good oral hygiene and dental care*: Interrogation revealed that these patients seldom if ever visited a dentist, and consequently, very few had restorations present. Oral examination usually showed large deposits of calculus, detritus, and food debris. Few, if any, of these patients had ever had thorough dental prophylaxis.

d) *Absence of certain nutritive requirements of the Lactobacillus acidophilus in the saliva*: Kniesner, Mann, and Spies (1) investigated the low incidence of dental caries from the point of view that a diet deficient in essential food substances for man may manifest itself by nutritional deficiencies in the saliva for the lactobacillus of the mouth. They found, however, that the amount of pantothenic acid in the saliva of these patients was sufficient to satisfy the growth requirement of the *Lactobacillus acidophilus*. Hill and Kniesner (4) extended these findings and reported that the minimum amount of pantothenic acid and dextrose in saliva necessary for the normal growth of the lactobacillus is far below the values we have found in the saliva of these patients.

e) *Effective amounts of fluorine in the water supply*: It is generally accepted that 1.00 ppm. of fluorine in the water supply ingested by an individual during the period in which the teeth are being laid down will confer a relative immunity to dental caries (5). With this in mind, the fluorine content of both well and spring water used by this group was investigated by Dr. Margaret L. Swain (1). The determinations made on 8 samples were found to range from 0.027 to 0.097 ppm., with an average of 0.049 ppm., an amount conceded as not high enough to be very effective.

f) *The relation of caries activity to the buffering capacity of saliva*: Many investigators have attempted to correlate dental caries activity with salivary functions and components. Fosdick, Hansen, and Epple (6) have made use of one of the saliva factors to formulate their chemical test for caries activity, and Hadley (7) has found that the *Lactobacillus acidophilus* content of the saliva may be used as a measure of caries susceptibility. Arnold and McLure (8), Becks (9), Becks, Wainwright, and Young (10), and Karshan (11), among others, have attempted to determine fundamental differences between susceptibility and immunity to dental caries among the salivary constituents, but their results have been, for the most part, inconclusive. A review of the literature brought out one factor that appeared significant, however; namely, the buffering capacity of the saliva. The relationship between the buffer capacity and caries activity has been one which, in one form or another, has held the attention of several previous workers in the field (12, 13, 14). Karshan (11), and Karshan, Krasnow and Krejci (15) have noted a definite relationship between the ability of saliva to neutralize acid and the caries activity in a given mouth. Fosdick (16) has pointed out that the buffering capacity of the saliva of the average caries immune patient is about 40% higher than that of the caries susceptible individual. The

results obtained from the investigation of the saliva buffering capacity and its relationship to the reduced dental caries activity warrant a separate report at this time.

MATERIALS AND METHODS

Selection of patients: Fifty patients with varying amounts of dental caries were arbitrarily selected following a medical, dental, and dietary history, supplemented by additional laboratory studies for caries activity. The medical history and physical examination entailed a search for signs and symptoms of nutritional deficiency diseases, and were made by Dr. Carl F. Vilter and Dr. Tom D. Spies. A dental history was taken which recorded the degree and location of redness of the mucous membrane, the periodontal condition of the individual teeth, the number of missing teeth, and the factors leading to their extraction. The dental examination was made with the exploring tine, mouth mirror, and full mouth or bite wing radiographs. A special examination of the angles of the mouth, tongue, skin, eyes, nose, and hair was made by both the dentists and physicians on the staff of the Nutrition Clinic. The dietary histories and their interpretations were made by Miss Jean Grant and Mrs. Dorcas Morgan. The base line values of caries activity were obtained from bacteriological and chemical tests which had been run prior to the start of this study, records of which were available in the Clinic files for each patient.

After all these observations were made, the patients were arbitrarily divided into 3 groups, the criteria being nutritional status and degree of caries activity. The 3 groups consisted of the following: Group I, 16 nutritionally deficient but relatively caries free patients; Group II, 20 well nourished patients with moderate caries activity; and Group III, 14 well nourished patients with rampant caries.

Method of testing the saliva: Thirty cc. of saliva stimulated by paraffin were collected from each patient for every examination. Three different methods were then used on each sample to test for caries activity. They were(a) the method of Fosdick, Hansen, and Epple (6), involving a chemical analysis for soluble calcium, for which 20 cc. of saliva were used, (b) Hadley's method (7) for determining the number of *Lactobacilli acidophilus* per cc. of saliva, which required 1 cc. of the sample, and(c) an unpublished method developed by the authors based upon the buffer capacity of the saliva, for which 5 cc. of the saliva sample were used. This buffering capacity was demonstrated by a titration curve against 0.1 N lactic acid. The 50 patients used on this study provided 154 samples of saliva. In performing the bacteriological test for dental caries activity, 5 instances of contamination with molds from the air were encountered so that in those instances the results of the bacteriological test were discarded.

A buffer is defined by Hitchcock as a "solution containing a weak acid or base with one of its salts. It therefore has reserve acidity and alkalinity and it can take up appreciable amounts of acid and alkali with only slight changes in pH."(17) Each buffer has a definite pH range at which it can act most effectively. The maximum efficiency of a buffer is obtained when $[H^+] = K_1$. This follows from the Henderson Hasselbach equation that $pH = pK_1 + \log \frac{[salt]}{[acid]}$.

The pK is related to the pH and is the negative log of the ionization constant K_1. For any given buffer couple the pK_1 remains practically constant. For example, the pK_1 is 6.1 for $BHCO_3:H_2CO_3$, and 6.8 for phosphates (18). It is generally regarded that the maximum efficiency of a naturally occurring buffer exists at the pH of the biological system in which it is normally found.

Starr (19) analyzed 610 specimens of human saliva obtained from 228 healthy normal subjects, and found the reaction to vary from pH 5.75 to pH 7.05. In 86% of the analyses variations were in a narrower range; namely, pH 6.35 to pH 6.85. Eisenbrandt (20) found the mean pH of unstimulated saliva of 7 subjects to be pH 6.64 $\pm$ 0.006 based upon 1,552 electronic tests made with the glass electrode at regular bi-hourly intervals for an entire year. On this basis, supplemented by the principle that a buffer is fairly efficient at any pH value not more than 1 unit removed from the pK_1 of the weak acid (17), we have assumed the pH of 6.0 to pH 7.0 to be indicative of optimum salivary pH, and to be the range at which the saliva buffers are most effective. Saliva samples differ, however, in their buffer capacities, which may be regarded as a measure of the concentration of the buffers. This difference was demonstrated by the use of titration curves secured for each sample of saliva studied by means of the following method:

The pH of the 5 cc. sample was measured with a Beckman pH meter. This instrument was also used for all subsequent determinations. Then, from a burette calibrated to deliver 0.04 cc. per drop, 0.1 N lactic acid was added drop by drop and a pH reading taken after the addition of each drop until a pH of 4.0 was reached. At this point the dissociation of 0.1 N lactic acid was itself suppressed because of the accumulation of lactate and hydrogen ions so that further addition of this acid had comparatively little effect (21). The readings were subsequently plotted to make a curve using pH changes as the ordinate and the cc. of 0.1 N lactic acid added to the sample as the abscissa. Finally, the sample of saliva which was incubated for 4 hours at 37°C in the Fosdick method was centrifuged, and the pH of the solid free centrifugate obtained. This result was included on the graph containing the corresponding titration curve, and denoted by the letter "A" (after incubation) (*fig. 1*).

OBSERVATIONS

Distinct differences between the 3 groups of patients used on this study were observed when the results of the 3 different tests for caries activity were compared (Table I). An analysis of the outcome of the buffer capacity test showed that Group I (16 nutritionally deficient patients whose teeth were relatively free from dental caries) had an average value of 0.615 cc. 0.1 N lactic acid; Group II (20 well nourished patients with moderate caries activity) had an average capacity of 0.484 cc. 0.1 N lactic acid; and Group III (14 well nourished patients with rampant caries) showed an average buffer capacity of 0.353 cc. 0.1 N lactic acid. It was noted that Group I had, as an average, a buffer capacity in the saliva 55.7% greater than that of the well nourished patients of Groups II and III combined. The results of the chemical test for caries activity were in

agreement with those of the buffer capacity test. The 16 patients in Group I, when divided on the basis of the Fosdick classification of caries activity, included 11 negatives, 1 plus/minus, and only 4 positives. The 20 patients in Group II comprised 5 negatives, 1 plus/minus, and 14 positives, while the 14 patients in Group III were all either 3 plus or 4 plus. The bacteriological test also provided corresponding results as the Fosdick negatives and plus/minus when combined, had a mean lactobacillus count of 3,200 per cc. of saliva, and the average of the Fosdick positives amounted to 24,600 bacteria per cc. of saliva.

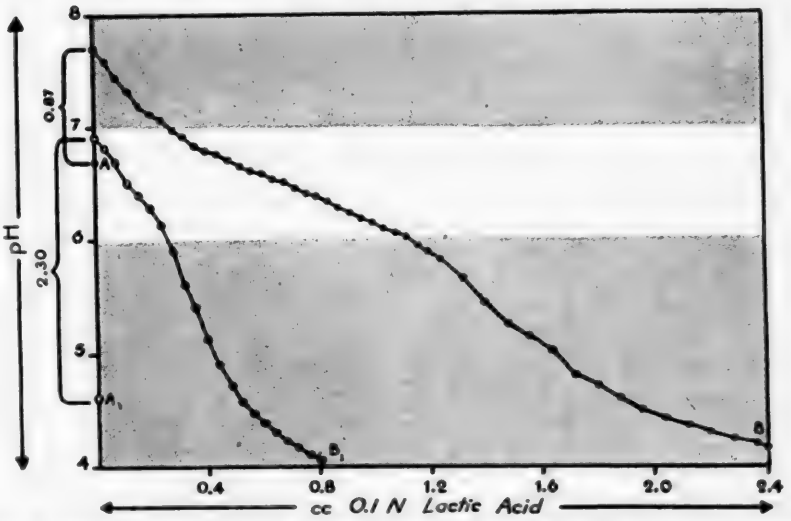

Fig. 1. Graph showing titration curves obtained when saliva from a caries immune patient and saliva from a patient with rampant caries were titrated against 0.1 N lactic acid.

●—● = Saliva from a caries immune patient. Chemical −. Buffer capacity (between pH 7.0–pH 6.0) 0.88 cc. 0.1 N Lactic Acid. Bacteriological 2,700/cc. Saliva.

○—○ = Saliva from a patient with rampant caries. Chemical ++++. Buffer capacity (between pH 7.0–pH 6.0) 0.2668 cc. 0.1 N Lactic Acid. Bacteriological 73,500/cc. Saliva.

A—pH Reading after incubation. B—pH Reading before incubation.

The observations summarized in Table II show the relationship of the results obtained with the 3 tests on the same sample of saliva, when they were plotted irrespective of the nutritional status of the patients. Of the 149 samples of saliva used for this purpose, 40 had a high buffer capacity and a low bacterial count. Thirty of the 40 were Fosdick negatives, 6 were plus/minus, and the remaining 4 were positives. (The preponderance of the negatives in this group suggests the inability of the relatively small number of bacteria to initiate and promote the carious process in the presence of a high concentration of buffers.) Fourteen of the 149 had a high buffer capacity and a high bacterial count. Seven of the 14 were negatives, 1 was plus/minus, and 6 were positive. (This

would indicate that in roughly 60% of the cases a high buffer concentration will offset the action of a high incidence of *Lactobacillus acidophilus*.) Sixty-eight of the 149 determinations had a low buffer capacity and a high bacterial count. All 68 were Fosdick positives. (The high number of bacteria appears to have been able to overcome the low buffering capacity of the saliva without any difficulty whatsoever.) Twenty-seven of the 149 tests showed a low buffer capacity and a low bacterial count. Twenty-six of the 27 were positive when tested by Fosdick's method. (It would appear that the small number of bacteria multiplied rapidly under optimum artificial conditions over the 4-hour period and

TABLE I

*The relationship between caries activity, as determined by chemical and buffer tests, and the nutritional status of fifty patients**

CLASSIFICATION OF CARIES ACTIVITY (FOSDICK)	GROUP I†		GROUP II‡		GROUP III§	
	No. of patients	Ave. no. cc. of 0.1 N lactic acid‖	No. of patients	Ave. no. cc. of 0.1 N lactic acid	No. of patients	Ave. no. cc. of 0.1 N lactic acid
−	11	0.719	5	0.680		
±	1	0.520	1	0.563		
+			1	0.593		
++	1	0.378	5	0.351		
+++	2	0.400	7	0.429	10	0.362
++++	1	0.240	1	0.285	4	0.330
Total.....	16		20		14	
Group ave. cc. of 0.1 N lactic acid..		0.615		0.484		0.353

* Table showing that the malnourished patients have the highest buffer capacity as well as the least activity as measured by Fosdick's method. Conversely, the well nourished patients have the lowest buffer capacity and the greatest amount of activity when measured by Fosdick's method.

† Nutritionally Deficient Patients, Caries Free

‡ Well Nourished Patients, Average Caries Activity

§ Well Nourished Patients, Rampant Caries

‖ Average number cc. of 0.1 N lactic acid refers to amount needed to lower pH of saliva sample from pH 7.0 to pH 6.0.

produced enough acid in the relative absence of buffers to raise markedly th- rate of formation of soluble calcium in the incubated sample.)

As seen in Table III where the results were tabulated according to the Fosdick classification of dental caries activity irrespective of the 3 groups of patients, an inverse correlation existed between the chemical activity and the buffer capacity for each sample of saliva. Those samples classified as Fosdick negative showed an average value of 0.710 cc. 0.1 N lactic acid with a range between 0.466 cc. and 0.960 cc. 0.1 N lactic acid (73.68% of these cases were in a narrower range, 0.630 cc.–0.790 cc. 0.1 N lactic acid), while those classified as Fosdick 4 plus had an average value of 0.330 cc. 0.1 N lactic acid with a range between 0.180 cc. and 0.500 cc. 0.1 N lactic acid (90.32% were in a narrower range,

0.250 cc.–0.410 cc. 0.1 N lactic acid). The intermediate positives and the plus/minus followed in line with these results.

TABLE II

*Relationship of buffer capacity to the Lactobacillus count and to the chemical activity in 149 samples of saliva**

CLASSIFICATION OF CARIES ACTIVITY (FOSDICK)	†HIGH BUFFER ACTION, §LOW BACTERIAL COUNT	‡LOW BUFFER ACTION, ‖HIGH BACTERIAL COUNT	HIGH BUFFER ACTION, HIGH BACTERIAL COUNT	LOW BUFFER ACTION, LOW BACTERIAL COUNT
−	30	0	7	1
±	6	0	1	0
+	2	0	2	1
++	1	9	1	15
+++	1	34	3	6
++++	0	25	0	4

* This table was arbitrarily divided into four parts. Values of 0.500 cc. 0.1 N lactic acid and below were regarded as indicative of a low buffer capacity, and those of 0.500 cc. 0.1 N lactic acid and above were regarded as indicative of a high buffer capacity. It was noted that these buffer values were relatively constant for samples taken from the same individual on different days. Lactobacillus counts of 5,000 and below per cc. of saliva were considered as low bacterial counts, and those above 5,000 per cc. of saliva were considered as high bacterial counts.

† Above 0.500 cc. 0.1 N lactic acid.

‡ Below 0.500 cc. 0.1 N lactic acid.

§ Below 5,000 per cc. of saliva.

‖ Above 5,000 per cc. of saliva.

TABLE III

*Comparison of buffer capacity with caries activity in 154 samples measured by Fosdick's method**

NO. OF SAMPLES	FOSDICK'S CLASSIFICATION OF CARIES ACTIVITY	RANGE OF CC. 0.1 N LACTIC ACID†	AVERAGE NO. OF CC. 0.1 N LACTIC ACID	PER CENT OF CASES WITHIN ±0.08 CC. 0.1 N. LACTIC ACID OF AVERAGE
38	−	0.466–0.960	0.710	73.68
8	±	0.400–0.900	0.642	62.50
5	+	0.320–0.640	0.544	80.00
27	++	0.240–0.540	0.389	86.67
45	+++	0.240–0.560	0.380	82.22
31	++++	0.180–0.500	0.330	90.32

* The buffer capacity correlates inversely with the chemical activity as measured by Fosdick's method.

† Number of cc. 0.1 N lactic acid refers to amount needed to lower pH of saliva sample from pH 7.0 to pH 6.0.

The results plotted in Table IV compared the buffer capacity and the decrease in pH during the 4-hour period of incubation, irrespective of the grouping of the patients. The Fosdick negatives were shown to have the smallest drop in pH, the average being 1.255 pH units with a range from 0.490 to 2.720 pH units.

(In 60.53% of the analyses variations were in a narrower range; namely, from 0.9555 to 1.555 pH units.) The Fosdick 4 plus had the largest decrease in pH, the average being 2.550 pH units with a range from 1.430 to 3.400 pH units. (In 67.74% of the cases variations were in a narrower range; namely, from 2.250 to 2.850 pH units.) The intermediate positives and the plus/minus followed in line with the observation that as the Fosdick value for caries activity increased, the drop in pH increased, and the buffer capacity was greatest in those instances where the pH drop was smallest. (The decrease in pH may be regarded as a function of the buffer concentration and the number of *Lactobacilli acidophilus* in a given sample of saliva.)

TABLE IV

*Comparison between caries activity and change in pH in saliva samples incubated for four hours at 37° C**

NO. OF SAMPLES	CLASSIFICATION OF CARIES ACTIVITY (FOSDICK)	AVERAGE DROP IN pH	RANGE OF pH	PER CENT OF CASES WITHIN ±0.3 pH UNITS OF THE AVERAGE
38	−	1.255	0.49–2.72	60.53
8	±	1.600	1.00–2.60	75.00
5	+	1.880	1.25–2.70	60.00
27	++	2.146	1.30–2.90	85.18
45	+++	2.377	1.00–3.30	73.33
31	++++	2.550	1.43–3.40	67.74

* The drop in pH may be regarded as a function of the Lactobacillus acidophilus count and the buffer capacity of the saliva in the sample incubated for four hours at 37° C.

DISCUSSION

A high correlation exists between the chemical, bacteriological, and buffer capacity tests for caries activity and the clinical findings. This correlation was demonstrated when the 3 tests were applied to 3 arbitrarily chosen groups of patients, divided on the basis of nutritional status and dental caries susceptibility. The nutritionally deficient patients (Group I), relatively free from dental caries, had a buffer capacity in their saliva 55.7% greater, as an average, than that of the well nourished patients with moderate or rampant caries activity (Groups II and III). The samples of saliva from the patients of Group I were negative in 73.33% of the cases while a similar result was obtained in only 15.12% of the samples supplied by the patients of Groups II and III combined when tested by the Fosdick method. The bacterial correlation was revealed when it was noted that the Fosdick negatives averaged 2,600 *Lactobacilli acidophilus* per cc. of saliva, and the positives averaged 24,600 per cc. of saliva.

The results obtained from this study, therefore, indicate that as the caries susceptibility (Fosdick, Hansen, Epple) increased, the buffer capacity (measured in terms of cc. 0.1 N lactic acid required to lower the pH of the saliva sample from pH 7.0 to pH 6.0) in the saliva decreased, and conversely, as the caries susceptibility (Fosdick, Hansen, Epple) decreased, the buffer capacity of the

saliva increased. The Fosdick activity bore a direct relationship to the lactobacillus count. By comparison, the lactobacillus count was inversely related to the buffer capacity of the saliva, such that a sample with a high buffer capacity had a low bacterial count, and a sample with a low buffer capacity in the saliva had a high bacterial count. The buffer capacity method made possible an explanation of the few seeming discrepancies between the results of the chemical and bacteriological tests on the same sample of saliva. Of the 149 samples of saliva investigated, seven showed a high bacterial count and a negative activity when tested by Fosdick's method, while 10 had a low bacterial count and a Fosdick activity which reached 3 plus in 3 cases and 4 plus in the other 4 cases. These apparent discrepancies may perhaps be explained by noting that the 7 samples with high bacterial counts and which tested negative by Fosdick's method also had a high buffer capacity in the saliva, indicating that the acid produced by the bacteria was rapidly neutralized. By contrast, the 10 samples with low bacterial counts and high activity by Fosdick's method had a low buffer capacity. This low concentration of buffers was probably unable to neutralize the acid produced by the small number of bacteria, causing enough acid to accumulate to give a highly positive result by Fosdick's method. It seems logical, therefore, to test for the buffer capacity of the saliva along with the other 2 methods, to determine more accurately the dental caries activity of any individual.

SUMMARY AND CONCLUSIONS

1. A definite and readily discernible difference exists between the buffer capacity of the saliva of a caries resistant and a caries susceptible individual as demonstrated by the study of 3 groups of patients arbitrarily chosen on the basis of nutritional status and previous caries activity. The results achieved with the buffer capacity method as described in this report correlate well with the chemical and bacteriological tests for caries activity on the same samples of saliva, as well as with the findings obtained with the mouth mirror, exploring tine, and dental x-ray. This buffer capacity test also offers an explanation of the few instances of noncorrelation between the chemical and bacteriological tests.

2. Malnourished patients, relatively free from dental caries, had the highest buffer capacity in the saliva, the lowest number of *Lactobacilli acidophilus*, and the least active Fosdick determinations. In contrast, the well nourished patients with either moderate or rampant caries showed a progressive decrease in the buffer capacity of the saliva and corresponding progressive increases in the lactobacillus counts and the Fosdick determinations.

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STUDIES OF THE EFFECT OF GLYCERITE OF HYDROGEN PEROXIDE UPON THE NUMBERS OF ORAL MICROORGANISMS[1]

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INTRODUCTION

The present paper is concerned with studies of the effects of an antiseptic solution upon bacteria normally inhabiting the mouth. Attention has been called to the fact that tests carried out in the mouth are even more difficult to interpret than those carried out on the skin (8). The Council of Dental Therapeutics of the American Dental Association has stated (13) "No method or combination of methods has been devised which will give a thoroughly satisfactory comparison of the germicidal agents in the test tube and under the conditions in which they are actually used".

The problems involved are complex. Bibby (2) has shown that the variations in bacterial flora in different parts of the same mouth, and the difference in the percentage of organisms in different mouths of the same group or series is so great that no significant relationship can be extablished between the oral flora and the state of oral health. His studies also demonstrate the fact that because of unknown dilution factors the number of bacteria in unit amounts of a mouth specimen is not significant, and that the only significant bacterial measurement which can be made is a determination of the percentage occurrence of the different types of micro-organisms independently of the absolute number of bacteria present. Crowley and Rickert (7) testing the effects of oral preparations conclude that count methods are not to be relied upon in testing mouth washes.

Although there is no laboratory test which parallels the conditions in the mouth, antiseptic substances which affect oral organisms can, to a certain degree, be tested by using them in the oral cavity. The number of bacteria discovered in samples before and after the use of such solutions may not be directly related to the total number of organisms present, but a marked, consistent change in type or a mathematically-significant decrease in mumbers may be considered due to the action of the antiseptic solution when control tests demonstrate no similar consistent changes. Antiseptic substances are recommended for oral antisepsis on the basis of date resulting from such tests. Appleton (1) stated: "No data

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are available which tell us how many bacteria are left in the mouth after carrying out these measures and perhaps it is impossible to obtain direct data of this sort. But we can obtain estimates of the number of bacteria removable in mouth rinsings after carrying out these measures and it is probably legitimate to assume that the number of bacteria left in the mouth is a function of the number removable in mouth rinsings."

Since the solution used in this study has only recently been described (4) its properties require a brief review. Its basic ingredient is hydrogen peroxide, the positive virtues of which are its well-known deodorant and detergent qualities, and its less-known hemostatic effects. It is non-toxic, non-irritating, and non-allergenic. On the other hand, hydrogen peroxide suffers from two obvious disadvantages. In the presence of peroxidase, its action is over so rapidly that the bacteria present are not affected; while in the absence of peroxidase, its action is so slow it has been denigrated with a low phenol coefficient. Under the proper circumstances, it has a broad anti-bacterial spectrum for both aerobic and anaerobic organisms.

One method of compensating for its disadvantages is to use hydrogen peroxide in a medium in which (in the presence of moderate amounts of peroxidase) its rate of decomposition will progress less rapidly. It occurred to Brown, Abramson, and Gorin (4) that the use of dry urea peroxide (4%) as a means of carrying hydrogen peroxide (1.5%) into a viscous anhydrous solvent, such as glycerol, would add to the virtues of peroxide solutions. Pure hydrogen peroxide, (100%), not obtainable when our work was initiated, can be used to make solutions of equivalent properties. The presence of the end-product urea (2.5%), a peptizing substance which not only hastens wound healing (12) but is also bacteriostatic (14), adds other positive virtues to the solution.

It was later discovered by one of us (EAB) that one explanation for the previously reported instability of such solutions was the presence of metallic trace elements in the glycerol. Oxine (8-hydroxyquinoline) added as an ingredient in quantities as little as 0.1% inactivated the metals and further stabilized the solution. It is interesting to note that Zentmyer (15) reported that oxine acted as an anti-bacterial agent because it made non-ionizeable salts of the metallic trace elements necessary to normal bacterial metabolism.

The solution used for the following experiments consists, therefore, of: hydrogen peroxide (1.5%) as derived from urea peroxide (4%) in anhydrous glycerol to which has been added oxine (0.1%). When this solution is diluted with an equal amount of sterile water for the experiments noted below, the concentration of hydrogen peroxide present is 0.75%.

Previous studies, using a modified agar cup plate method (5) to compare the solution with the tinctures of iodine (7%) , Metaphen (0.5%), Mercresin (standard strength), Zephiran (0.1%), and Phemerol (0.1%), as well as aqueous Mercurochrome (2%), against 6 Gram positive and 5 Gram negative organisms show that, under the test conditions, it approximates tincture of iodine in its effects. It demonstrates bactericidal power for *Staphylococcus aureus*, streptococci (alpha and beta haemolytic), an enterococcus, *Micrococcus epidermidis*, *Proteus mira-*

bilis, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aerobacter aerogenes*, and *Eberthella typhosa*, and bacteriostatic power for the diphtheroids.

Tissue tolerance studies (3) by patch tests for 48 hours on 100 normal individuals, and an examination of over 750 patients upon whom the solution had been used for the treatment of acute and chronic infections, demonstrated a likelihood at the 95% level that irritation or allergenicity would be expected in less than 1% of the general population.

Since peroxide solutions by virtue of their non-toxicity are adaptable for use in the mouth, it was suggested by one of us (L. W. S.) that studies of the effects upon oral bacteria be done. Such experimental work would seem to be justified by the previous studies in this field. Farrell and McNichols (9) suggest the employment of hydrogen peroxide in conjunction with any or all medications for the treatment of Vincent's stomatitis. Meleney (11) and also Mallet and Guralnick (10) have reported clinical results with zinc peroxide solutions.

EXPERIMENTAL METHODS

For the collection of samples from the mouth, a modification of the method described by Bryan (6) was used. A sterile toothbrush is dipped into 10 ml. of sterile water, and the teeth brushed vertically for 2 minutes. The toothbrush is rinsed in the remainder of the sterile water after 1 minute of brushing, and again at the end of the brushing period. After collecting the saliva in the collection tube, the mouth is then rinsed vigorously for 1 minute with 10 ml. of sterile water and this rinsing added to the material collected from the toothbrush. The total volume is adjusted to 20 ml. Samples for darkfield examinations are also collected from the gingival crevices with pointed wooden applicators.

After shaking for 10 minutes on a Kahn shaking machine, the 20 ml. samples are plated out in the following dilutions and media. For the general types of mouth bacteria, dilutions of 1:1,000, 1:10,000 and 1:100,000 in sterile water were plated in tryptone glucose extract agar, and the colonies on the plates counted after incubation at 37°C. for 2 days. This medium contains 0.5% tryptone, 0.1% glucose, 0.5% beef extract, and 1.5% agar adjusted to a pH of 7.0. For the determination of the numbers of lactobacilli, dilutions of 1:10 and 1:100 are plated in neopeptone tomato juice agar containing 40% filtered tomato juice, 1% peptone, 1% peptonized milk, and 2% agar adjusted to a pH of 6.0. The cultures are incubated in an atmosphere of carbon dioxide (10%) for 2 days at 37°C.

The numbers of fusiform bacteria are determined by plating 1:10 and 1:100 dilutions of the samples in crystal violet potato extract agar. This medium contains: 2% tryptose; 0.3% beef extract; 0.05% cysteine hydrochloride; 0.1% glucose; 0.01% crystal violet potato extract, and 2.0% agar at a pH of 7.6.

EXPERIMENTAL DATA

1. *The diminution in the number of oral bacteria following 1 application of glycerite of hydrogen peroxide (0.75%).* Six subjects with normal mouths were used. Samples of saliva were collected from each subject twice weekly for a

period of 3 weeks, both before and at various times after the application of the germicidal solution. One-half hour after samples were collected to determine the normal count, the teeth and gingival crevices were brushed for 2 minutes with 4 ml. of the hydrogen peroxide solution (1.5%) described above, after diluting with an equal volume of sterile water. Immediately following this the mouth was carefully rinsed for 1 minute with 10 ml. of the same solution. At successive intervals of 10 minutes, 30 minutes, 1 hour, and 2 hours after treatment, samples were collected and tested as described above.

TABLE I

Total number of bacteria removed from mouth as determined by plating in tryptone glucose extract agar (average of 6 different tests)

SUBJECT	INITIAL COUNT (NORMAL)	COUNT AFTER USE OF GLYCERITE OF HYDROGEN PEROXIDE (0.75%)				PERCENTAGE DECREASE AFTER 2 HOURS
		10 minutes	30 minutes	1 hour	2 hours	
1	13,850,000	2,665,000	2,375,000	1,305,000	1,100,000	93%
2	20,950,000	3,090,000	1,920,000	6,200,000	7,100,000	66%
3	5,130,000	1,230,000	1,470,000	2,470,000	3,820,000	26%
4	16,625,000	7,340,000	1,180,000	4,725,000	6,305,000	69%
5	12,762,000	2,571,000	3,825,000	6,400,000	2,390,000	81%
6	9,250,000	6,265,000	5,935,000	6,708,500	2,655,000	71%

TABLE II

Total number of fusiform organisms removed from mouth (average of 6 different tests)

SUBJECT	INITIAL COUNT (NORMAL)	COUNT AFTER USE OF GLYCERITE OF HYDROGEN PEROXIDE (0.75%)				PERCENTAGE DECREASE AFTER 2 HOURS
		10 minutes	30 minutes	1 hour	2 hours	
1	1,980	820	575	640	840	57%
2	290	210	150	160	140	52%
3	40	120	60	60	40	0%
4	75	65	20	20	17	77%
5	325	130	80	85	220	93%
6	1,335	365	270	177	110	91%

The results given in the Table I indicate the diminution in the total number of organisms following a single application of the solution as determined by plating out samples in tryptone glucose extract agar. The numbers represent an average of 6 different tests made twice weekly over a 3-week period. The reduction varied from 26% to 93% of the total number of bacteria normally present. Control tests showed that there was no reduction in the number of bacteria in the mouth due to the brushing and rinsing technique with sterile water, the numbers being as high one-half hour after collecting samples as they were initially.

Samples plated on crystal violet potato extract agar (Table II) demonstrated a reduction varying from 52–93% in the number of fusiform bacteria. In 1 subject with few oral fusiform organisms, there was no apparent reduction in numbers. The lactobacilli were evidently unaffected by the solution, since plate

counts were as high after as they were before treatment. However, lactobacilli were detected in the mouths of only 2 of the 6 subjects tested, and further tests are necessary before final conclusions can be made.

Darkfield examinations of scrapings from the gingival crevices before and after treatment showed a marked germicidal action of the solution for the spirochete and fusiform organisms. Three of the 6 subjects presented large numbers of spirochete organisms in the gingival scrapings. In all cases, in 1 to 2 hours after treatment, these organisms disappeared and very few could be found after 6 hours. After 1 treatment, however, they were generally present again in large numbers in 24 hours. A definite reduction in the number of fusiform bacteria was also demonstrated after the use of the hydrogen peroxide solution.

2. *The diminution in the number of oral organisms following the brushing of the teeth with glycerite of hydrogen peroxide (1.5%) and rinsing the mouth with* glycerite *of hydrogen peroxide (0.75%) morning and night.* Eleven subjects with normal mouths were used in this study. Three of these subjects continuously demonstrated large numbers of oral fusiform and spirochete bacteria. To determine the normal oral flora of this group, saliva was collected by the brushing and rinsing technic, and plated in tryptone glucose extract agar as described above. Scrapings on wooden applicators were also obtained for darkfield examination.

After the normal microbic flora was determined, 6 of the subjects discontinued the use of their usual dentifrice and began a program of brushing their teeth and rinsing their mouths with the hydrogen peroxide solution for an 11-day period. The teeth were brushed twice daily with glycerite of hydrogen peroxide (1.5%), adding 2 ml. to the brush for each application and brushing the teeth for approximately 1 minute. The mouth was then rinsed with 10 ml. of this solution after diluting with an equal volume of sterile water.

For comparative and control purposes, 3 other subjects were asked to dissolve orally 1 penicillin lozenge (1000 units) 3 times daily, using 1 lozenge about 1 hour after each meal. One subject rinsed his mouth with 10 ml. of undiluted Listerine after brushing his teeth, and 1 control subject brushed his teeth as usual without the use of any medication during the study period. Samples were collected from all subjects at 11:00 A.M. each day during the time of the experiment.

The numbers of bacteria found in the mouth before and after the use of the medications, as determined by plating out the samples in tryptone glucose extract agar, are recorded in Table III. The percentage decrease varied from 11.3 per cent to 79.1 per cent when the glycerite of hydrogen peroxide was used, and from 40.6 per cent to 86.7 per cent when the penicillin was employed. Rinsing the mouth with Listerine did not appreciably decrease the number of bacteria; and also, in the control subject, there was no diminution in the number of organisms during the time of the study. It should be noted that all counts are based on samples collected at 11:00 A.M. and not immediately after the solutions were used.

The results of direct examination by darkfield microscope indicated that there was a definite reduction in the number of spirochete and fusiform bacteria following the use of the glycerite of hydrogen peroxide and the penicillin, particularly in those subjects normally harboring large numbers of these organisms.

3. *The diminution in the number or oral organisms following the rinsing of the mouth with glycerite of hydrogen peroxide (0.75%).* The following tests were made to determine the effect on the oral bacteria of several applications of the hydrogen peroxide solution. For this purpose, the mouth and throat were rinsed for 1 minute with 10 ml. of the glycerite of hydrogen peroxide (0.75%) 4 times at intervals of 1 hour.

Samples were collected from the mouth by the brushing and rinsing technic previously described except that during the rinsing process the throat was also gargled with the rinsing solution. Samples were obtained before using the medi-

TABLE III

Diminution in number of oral bacteria following daily use of glycerite of hydrogen peroxide and other medications for period of 11 days determined by plating in tryptone glucose extract agar

SUBJECT	MEDICATION	NORMAL COUNT. AVEG. OF 5 TESTS MADE AT 1–2 DAY INTERVALS OVER 1-WEEK PERIOD	COUNT DURING MEDICATION PERIOD. AVEG. OF 4 TESTS MADE AT 2–3 DAY INTERVALS OVER 11-DAY PERIOD	PER CENT DECREASE
1S	Glycerite of hydrogen peroxide	241,200,000	214,000,000	11.3%
2F	Glycerite of hydrogen peroxide	265,500,000	55,700,000	79.1%
3P	Glycerite of hydrogen peroxide	85,720,000	72,400,000	15.6%
4Sh	Glycerite of hydrogen peroxide	93,600,000	69,000,000	26.3%
5Car	Glycerite of hydrogen peroxide	145,000,000	64,260,000	55.7%
6C	Glycerite of hydrogen peroxide	133,400,000	108,000,000	18.5%
7B	Penicillin lozenges	122,000,000	72,500,000	40.6%
8G	Penicillin lozenges	107,500,000	14,400,000	86.7%
9M	Penicillin lozenges	188,000,000	55,000,000	70.8%
10Pi	Listerine	101,000,000	100,000,000	0.1%
11J	Control	114,225,000	123,000,000	0%

cation, and then every half hour following a mouth rinse with the solution. Samples were also collected 2 and 4 hours following the last rinse.

To determine whether the number of bacteria might be mechanically reduced by the hourly brushing and rinsing process, control experiments were made by collecting the samples as described above after rinsing the mouth with 10 ml. of sterile water 4 successive times at intervals of 1 hour.

The samples were plated out in tryptone glucose extract agar, and the colonies counted after incubation at 37°C for 2 days. Three subjects were used for this study, and tests were made on 2 different days both for the control and the glycerite of hydrogen peroxide determinations.

The results obtained are summarized in Tables IV and V. As the data in Table IV indicate, the number of bacteria in the mouth and throat were not mechanically reduced by the brushing and rinsing technic employed for this

study. However, when the mouth and throat were rinsed with 10 ml. of the hydrogen peroxide solution (0.75%), a very marked diminution in the number of bacteria occurred (Table V). In all cases, there was a continual decrease after each application of the solution, the greatest reduction in numbers occurring one-half hour after the last treatment. The number of bacteria generally began

TABLE IV

As control test a pre-rinse count was taken at 9:55 A.M. The subjects rinsed the mouth with sterile water at 10, 11 and 12 A.M. and 1 and 2 P.M. The numbers of organisms are listed in millions

SUBJECTS.....	1	2	3	4	5	6
Sample at 9:55 am	9.8	20.0	11.7	10.8	22.9	13.2
10:30 am	9.3	7.8	5.0	4.5	19.6	13.8
11:30 am	6.5	9.2	7.1	8.8	14.5	14.6
12:30 am	9.4	21.0	11.3	11.8	21.5	16.0
1:30 pm	7.9	16.9	5.2	7.2	13.9	17.2
2:00 pm	15.0	14.1	12.3	8.7	38.4	16.8
3:00 pm	12.9		8.8		41.2	

TABLE V

To determine effects of glycerite of hydrogen peroxide (0.75%) pre-rinse sample was taken at 9:55 A.M. Subjects rinsed mouth with solution at 10, 11 and 12 A.M. and 1 P.M. Sample estimations were made at 10:30, 11:30 and 12:30 A.M., and 1:30, 2:00 and 3:00 P.M. The numbers of organisms are listed in millions.

SUBJECTS.....	1	2	3	4	5	6
Sample at 9:55 am	24.0	17.8	22.0	6.6	25.6	25.5
10:30 am	8.2	7.4	8.1	3.4	11.2	7.6
11:30 am	6.6	3.2	6.0	3.0	11.0	2.4
12:30 am	6.2	3.6	3.8	2.2	4.6	1.4
1:30 pm	4.1	2.7	2.6	1.4	3.0	1.2
2:00 pm	6.2	5.4	4.9	3.0	5.0	1.8
3:00 pm	12.0	5.4	5.8	2.4	11.8	1.6

to show an increase 1 and 2 hours after the last application of the glycerite of hydrogen peroxide solution.

SUMMARY

Glycerite of hydrogen peroxide solutions (1.5% and 0.75%) used as a dentifrice and as a rinse demonstrate an appreciable bactericidal action upon oral microorganisms. This action was particularly marked for the oral spirochetes.

Direct examination of material from the teeth and gingival crevices by the

darkfield technic would seem to be an effective supplementary method for studying the action of antiseptic solutions on spirochetal organisms.

The decrease in the numbers of oral micro-organisms continues while the solution is being used, and tends to return to higher counts soon afterwards.

The effects of solutions of glycerite of hydrogen peroxide upon oral infections will be reported upon in the near future.

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CHEMICAL STUDIES IN PERIODONTAL DISEASE III

Putrefaction of Salivary Proteins[1]

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It has been shown that saliva from individuals suffering from periodontal disturbances will putrefy more rapidly than the saliva from individuals with normal periodontal tissues (1). The small differences noted were obtained by methods that determined only the increase in carboxyl groups and the increase in malodorous substances, and hence may not be a true measure of the actual rate of putrefaction.

In the process of putrefaction, the protein molecule is probably first hydrolyzed to amino acids, after which the amino acids undergo decarboxylation, a deaminization and certain oxidation-reduction reactions (2). When a protein is hydrolyzed, each peptide linkage yields an amino group and a carboxyl group. Thus, if no further actions were involved, the formol titration, which determines the number of free carboxyl groups, would be an accurate indication of the amount of hydrolysis that had occurred. However, decarboxylation, deamination and oxidation-reduction reactions also occur. Furthermore, it has not been definitely established whether these reactions occur according to a definite sequence, or whether they may occur simultaneously. If the rate of hydrolysis were faster than the decarboxylation, there would be an accumulation of carboxyl groups and the formol titration would yield quite accurate results. If decarboxylation were faster than the hydrolysis, little change in the number of carboxyl groups would occur and the hydrolysis could not be estimated by this method.

Of the amino acids progressively formed by hydrolysis during putrefaction, only a few, such as tyrosine and tryptophane, can be rapidly and accurately estimated in saliva by available methods. However, certain degradation products, such as indole from tryptophane, phenol from tyrosine, and sulfides from the sulphur-bearing amino acids, can be estimated, thus frunishing a rough measure of the amount of decarboxylation, deaminization and oxidation-reduction. Furthermore, many compounds formed by these latter reactions, such as cadaverine, skatole and sulfides, are malodorous, and hence may be detected in small quantities by means of the osmoscope. Mucin is rich in amino acids which yield these compounds (3).

In the present continuation of the earlier work, the rate of putrefaction of the salivary proteins was measured by means of the formol titration and by estima-

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tion of indole, tryptophane, sulfides, pH, pO and oxidation-reduction potentials. It was thought that with this additional data, much of which is interdependent, a more accurate estimation of the rate of putrefaction would be secured.

METHODS

The saliva for this investigation was collected from selected patients of the Northwestern University Dental School Clinic, who were classified according to their periodontal conditions as estimated on the basis of measurement of periodontal pockets in the mouth and on the basis of the apparent amount of bone resorption as indicated by radiographs. Patients whose mouths showed no apparent tendency to pocket formation were selected for Group 1. This group also included those individuals in whose mouths gingival recession had kept pace with the bone recession so that no pockets had formed. A few shallow pockets or early bone resorption around a few teeth placed the patient in Group 2. A few deep pockets or general but moderate loss of bone classified the patient as Group 3. Group 4 consisted of those individuals in whom there were many deep pockets and extensive loss of bone. Although the classification of the patients was based on the above, all conditions of the mouth, such as caries susceptibility, the type of dental restorations, the probability of occlusal trauma and other conditions, such as infected third molar opercula, were noted and recorded.

It is realized that this classification is purely subjective, depending upon the judgement of the examiner; however, as all of the examinations were made by the same individual, variations were minimized. Furthermore, as the patients were grouped on the basis of both clinical and radiographic evidence, errors in judgement were further minimized.

The rate of putrefaction was determined both aerobically and anaerobically. Furthermore, as some preliminary results indicated that the amount of protein in the saliva may be a limiting factor, the amount of substrate was increased by the addition of casein.

It was impossible to secure sufficient saliva at any one time from one individual for all 4 types of incubation, so a separate sample was collected for each type of incubation; but, as far as was possible, all 4 types of incubation were performed at different times on samples of saliva from the same individual. When the saliva was incubated anerobically in the presence of casein, smaller samples could be used, thus making it unnecessary to collect such large amounts of saliva for these tests. The appropriate amount of saliva (30–50 cc.) was collected in a clean, sterile flask by means of paraffin stimulation. The saliva was immediately incubated; and at 1, 3 and 24 hour intervals, samples were removed for analysis.

Aerobic incubation: The cotton-stoppered collection flask, containing 45–50 cc. of freshly collected saliva, was placed in an incubator at 37.5°C. At 1, 3 and 24 hour intervals, 15 cc. samples were removed for analysis.

Aerobic with casein: Immediately after the sample was collected, 15 cc. portions of saliva were added to each of 3 50 cc. screw cap vials, to which had been added 150 mg. of casein. The caps were then securely placed and the vials were fastened to a mechanical shaker and kept in motion while being incubated at 37.5°C.

At 1, 3 and 24 hour intervals a vial was removed and its contents analyzed. Constant shaking was found necessary to insure complete availability of the casein.

Anaerobic incubation: The freshly collected saliva was divided into 3 15 cc. portions and placed in 20 cc. screw cap vials. Sterile glass beads were added until each vial was full. The cap was then placed on and the vial was mechanically rotated during the incubation. Care was taken so that in no case was an air bubble present during the incubation. The motion of the glass beads provided mixing. At 1, 3 and 24 hour intervals, vials were removed and the contents analyzed.

CHEMICAL PROCEDURES

Each sample that was removed at each interval was analyzed for free carboxyl groups, indole, tryptophane and sulfides. The pH, pO and oxidation-reduction potential were determined and the rH and EH were calculated.

Free carboxyl groups: The free carboxyl groups were determined by means of the formol titration according to Sorensen (4), using 1 cc. of saliva and 0.01 N NaOH or H_2SO_4 (+) to bring the sample to the neutral point, using phenolphthalein as the indicator. One cc. of neutral formalin was then added and the solution was titrated with 0.01 N NaOH to phenolphthalein. The results are expressed in cc. of 0.01 N NaOH required to neutralize 1 cc. of saliva.

Analysis for indole: The method of Chernoff (5) was adapted for salivary analysis. Five cc. of saliva for the 1 and 3 hour samples, except those incubated anaerobically with casein, and 3 cc. for the 24 hour and 3 hour samples incubated anaerobically with casein was accurately pipetted into 125 cc. separatory funnels with 10 cc. of purified chloroform. The mixture was shaken mechanically for 2 minutes. The mixture was allowed to separate and the chloroform was drawn off into 50 ml. Erlenmeyer flasks. Another extraction was made with 10 ml. of chloroform and the combined extracts transferred to another clean, dry separatory funnel. The Erlenmeyer flask was rinsed with 5 cc. of chloroform and this too was added to the extracts. The water layer was saved for tryptophane analysis. Two ml. of a 0.2% solution of p-dimethylaminobenzaldehyde in 85% phosphoric acid were added to the 25 cc. of chloroform extract and the mixture was shaken for 2 minutes, after which 10 cc. of glacial acetic acid were added and the mixture again shaken for 30 seconds. The mixture was allowed to separate, leaving a colored, acid bottom layer. This was drawn off into a 10 ml. volumetric flask. The chloroform was washed with 1 cc. of 85% phosphoric acid, after which the bottom layer was added to that in the 10 ml. flask. The flask was filled to the 10 ml. mark with glacial acetic acid and the color concentration was read on a Coleman spectrophotometer at $\lambda = 550\ \mu\mu$. The reading was compared with a standard curve. The results are expressed as micrograms of indole per 1 cc. of saliva.

Tryptophane analysis: The water layer from the first chloroform extraction of the indole analysis was used for the analysis of tryptophane according to the method of Boyd (6). The saliva, after the chloroform extraction, was centri-

fuged and 1 cc. of the clear supernatant fluid was used for the analysis. The results were expressed as micrograms per cc. of saliva.

Determination of sulfide: The sulfide determination was done by the method of Lorant (7), in which p-aminodimethyl-aniline is converted to methylene blue which is determined colorimetrically. It was found that there was something in the saliva that interfered with the production of methylene blue, which necessitated the precipitation of the sulfide. Five cc. of saliva were pipetted into 150 mg. of zinc oxide and 1 cc. of concentrated borax solution in a glass stoppered test tube. The contents were well mixed to precipitate the zinc sulfide, after which the mixture was centrifuged. The clear supernatant fluid was poured off and the tube inverted to drain. At this time, 2 cc. of the zinc reagent, 5 cc. of distilled water, 1.5 cc. of p-aminodimethyl-aniline and 0.4 cc. of ferric ammonium sulphate were added. The mixture was stoppered and shaken. After 1 hour, 0.2 cc. of ethyl alcohol was added to destroy the foam and the mixture was centrifuged. The clear blue supernatant fluid was transferred to a Coleman spectrophotometer and the transmission read at λ 660 $\mu\mu$. The results are expressed in micrograms per cc. of saliva.

The pH, pO and oxidation-reduction potential: Insofar as the measurements of pH, pO and oxidation-reduction potential do not contaminate the saliva, these measurements are taken on the original samples before the other analyses are made. The pO was measured by means of the osmoscope (8). The pH was measured by means of a glass electrode and a Coleman vacuum tube potentiometer. The oxidation-reduction potential of the cell (Ec) was measured by the same instrument using gold as the inert electrode. The electrode potential, EH, was calculated by the equation $EH = Ec + 242$. The variation of potential due to pH was eliminated by the calculation of rH where $rH = 2pH + \frac{EH}{30}$, where EH is expressed in millivolts.

The results of each determination were recorded and subjected to statistical analysis. The results of the various groups were compared and the critical ratios of Groups 1:2, 2:3, 1:3, 3:4, 1:4 and 2:4 were calculated. The number of cases, mean, standard deviation from the mean, per cent within this range, total range and the critical rates are shown in Tables I to X inclusive.

DISCUSSION AND RESULTS

pH: In all cases the pH of the 1 hour sample was within the normal range of saliva. In all cases where the saliva was incubated aerobically, the reaction became more alkaline with incubation. This may indicate a progressive decarboxylation or a loss of CO_2. When the samples were incubated anaerobically, this increase in alkalinity was not observed, which probably indicates that the change under aerobic incubation is due to a loss in CO_2. However, it does not mean that decarboxylation did not occur under anaerobic conditions, as any CO_2 formed by decarboxylation would remain in solution. Perhaps an analysis for CO_2 would yield valauble information. In any event, an inspection of the critical ratios shows that in only scatteted instances were the results significant if we assume a critical ratio of 3 or more as significant.

The oxidation-reduction potential, Ec, EH and rH: The oxidation-reduction potential, Ec, in all cases showed a marked variation, as indicated by the wide variation from the mean. This was true at all time intervals and under all methods of incubation. In many cases the potential at the 1 hour interval was plus

TABLE I

pH

INCUBATION:		AEROBIC			AEROBIC + CASEIN			ANAEROBIC			ANAEROBIC + CASEIN		
Group	Hours:	1	3	24	1	3	24	1	3	24	1	3	24
I	Cases	3	6	6	4	4	4	3	3	3	4	4	4
	Mean	6.75	7.16	8.06	5.82	6.10	6.39	7.26	7.12	6.94	6.40	6.18	6.33
	σ	0.36	0.52	0.46	0.41	0.43	0.09	0.32	0.36	0.49	0.48	0.38	0.15
	Range	6.31–7.20	6.53–7.85	7.50–8.70	5.30–6.40	5.75–6.82	6.25–6.50	6.83–7.56	6.50–7.43	6.23–7.41	5.78–6.92	5.52–6.50	6.18–6.55
	Per cent	33	50	67	50	75	50	100	67	67	50	75	75
	c.r. 1:2	4.90	3.66	1.04	1.86	1.43	3.76	0.63	0.52	0.72	0.04	0.86	1.27
II	Cases	23	33	33	9	9	7	17	18	17	23	23	23
	Mean	7.62	7.75	8.52	6.18	6.38	7.02	7.39	7.03	6.77	6.41	6.31	6.41
	σ	0.25	0.23	0.25	0.45	0.43	0.69	0.79	0.29	0.24	0.28	0.25	0.16
	Range	6.91–8.20	7.30–8.27	7.92–8.96	5.30–6.80	5.70–7.05	6.09–8.10	6.82–7.76	6.36–7.49	6.21–7.23	5.80–6.99	5.70–6.95	6.02–6.65
	Per cent	91	79	67	56	56	57	100	61	71	74	65	61
	c.r. 2:3	1.01	0.06	0.43	0.70	0.34	4.06	1.59	2.67	0.97	1.29	0.29	0.97
III	Cases	18	14	13	6	6	4	10	9	10	13	12	11
	Mean	7.55	7.75	8.55	6.26	6.35	6.33	7.17	6.89	6.73	6.50	6.33	6.38
	σ	0.35	0.35	0.35	0.17	0.11	0.09	0.40	0.27	0.19	0.30	0.29	0.12
	Range	6.90–8.20	6.96–8.24	7.22–9.00	5.94–6.46	6.20–6.52	6.19–6.42	6.30–7.65	6.20–7.20	6.46–7.01	5.75–7.02	5.55–6.71	6.16–6.60
	Per cent	78	64	77	67	67	75	60	78	60	77	75	73
	c.r. 1:3	4.39	3.46	1.11	2.50	1.41	0.41	0.59	1.32	0.91	0.52	0.94	0.73
	c.r. 3:4	0.83	2.01	0.23	2.83	2.51	0.61	4.58	3.60	4.47	4.47	3.03	2.27
IV	Cases	4	6	5	6	5	3	3	3	3	9	5	4
	Mean	7.47	7.52	8.53	5.91	6.13	6.28	6.88	6.71	6.50	6.06	5.91	6.14
	σ	0.20	0.30	0.15	0.37	0.23	0.18	0.25	0.21	0.22	0.33	0.37	0.26
	Range	7.15–7.70	7.18–7.95	8.35–8.74	5.50–6.35	5.80–6.38	6.15–6.53	6.55–7.16	6.44–6.94	6.19–6.67	5.63–6.59	5.30–6.32	5.80–6.50
	Per cent	50	67	67	33	60	67	33	33	67	67	60	50
	c.r. 1:4	3.79	1.99	1.07	0.47	0.18	1.15	2.34	2.29	1.84	1.79	1.37	1.63
	c.r. 2:4	1.73	2.32	0.20	1.72	1.93	3.99	3.47	4.73	4.18	3.90	3.06	2.94

and in some cases it was minus, but in all cases there was a gradual decrease so that at the 24 hour interval the potential was always negative. In only scattered instances were the critical rates significant. When EH, or single electrode potential, was calculated, no increase in significance was observed; and when the variation in pH was eliminated, rH of the potential was still not significant. It is thought that although at present no significance can be placed on these measurements, they may be of considerable importance.

TABLE II

*Ec**

INCUBATION:		AEROBIC			AEROBIC + CASEIN			ANAEROBIC			ANAEROBOI + CASEIN		
Group	Hours:	1	3	24	1	3	24	1	3	24	1	3	24
I	Cases	3	6	6	4	4	4	3	3	3	4	4	4
	Mean	+6.7	+27.7	−8.2	+2.5	+1.3	−206.3	+36	−57	−184	+11	−35	−175
	σ	27.18	25.54	24.63	18.20	23.82	25.59	53.01	81.48	289.10	37.33	116.3	83.56
	Range	+45–	+66–	+27–	+25–	+25–	−165–	+76–	+59–	−139–	+67–	+130–	−60–
		−15	−10	−45	−20	−25	−230	+140	−185	−250	+30	−160	−275
	Per cent	67	67	67	50	75	100	100	33	100	50	50	50
	c.r. 1:2	0.56	7.21	4.36	0.15	2.45	0.74	1.79	2.37	0.42	0.35	0.91	2.21
II	Cases	23	33	34	9	9	7	17	18	17	23	23	23
	Mean	−2.3	−55.0	−52.6	−0.7	+29.7	−219	−14	−154	−242	+17	−78	−248
	σ	64.49	70.87	59.11	86.14	35.99	59.55	66.38	78.01	23.60	82.15	103.82	34.16
	Range	+61–	+103–	+43–	+110–	+86–	−78–	+78–	+59–	−200–	+140–	+130–	−126–
		−179	−240	−180	−120	−20	−260	−136	−256	−288	−160	−208	−278
	Per cent	91	70	53	44	44	86	47	72	65	61	74	83
	c.r. 2:3	0.81	1.22	0.41	0.76	4.74	1.25	0.52	0.95	1.17	7.49	4.73	1.71
III	Cases	18	14	13	6	6	4	11	9	10	13	12	11
	Mean	−25.3	−80.1	−46.6	−16.3	−38.8	−242.8	−37	−175	−252	−98	−164	−262
	σ	79.64	100.56	65.54	6.63	54.44	32.14	209.13	73.52	35.51	50.62	50.46	32.17
	Range	+91–	+58–	+16–	−8–	+10–	−200–	+50–	−14–	−199–	−22–	−56–	−210–
		−255	−255	−250	−25	−150	−290	−150	−275	−295	−192	−236	−299
	Per cent	72	57	92	50	83	50	100	78	70	69	67	55
	c.r. 1:3	1.19	5.30	2.60	2.56	2.16	2.28	1.43	2.76	0.49	6.19	2.78	2.62
	c.r. 3:4	0.05	1.62	1.11	1.20	1.51	1.57	1.84	3.80	0.53	1.60	0.99	0.11
IV	Cases	4	5	4	6	5	3	3	3	3	9	5	4
	Mean	−13.0	−156.4	−30.5	−63.0	−100.8	−275.3	+54	−106	−239	−114	−125	−263
	σ	113.73	128.00	17.84	128.96	111.79	34.66	45.64	9.76	42.35	94.63	111.44	16.72
	Range	+120–	−30–	−12–	+150–	+100–	−239–	+106–	+22–	−196–	+90–	+95–	−245–
		−163	−363	−55	−238	−239	−322	−5	−126	−296	−244	−200	−290
	Per cent	50	80	50	67	60	33	33	33	66	56	80	50
	c.r. 1:4	0.43	4.20	2.20	1.66	2.63	3.58	0.55	1.24	0.39	8.56	1.54	2.66
	c.r. 2:4	0.24	2.30	2.24	1.42	1.51	1.57	2.77	3.56	0.13	11.07	1.17	1.89

* Results expressed as millivolts.

Results expressed in millivolts.

TABLE III

*EH**

INCUBATION:		AEROBIC			AEROBIC + CASEIN			ANAEROBIC			ANAEROBIC + CASEIN		
Group	Hours:	1	3	24	1	3	24	1	3	24	1	3	24
I	Cases	3	6	6	4	4	4	3	3	3	4	4	4
	Mean	+248.7	+269.7	+233.8	+244.5	+243.3	+35.8	+278	+185	+58	+253	+207	+67
	σ	27.18	25.54	24.63	18.20	23.82	25.59	53.01	81.48	289.10	37.33	116.30	83.56
	Range	+287– +227	+308– +232	+269– +197	+267– +222	+267– +217	+77– +12	+318– +202	+301– +57	+103– −8	+309– +212	+372– +82	+182– −33
	Per cent	67	67	67	50	100	75	67	33	100	50	50	50
	c.r. 1:2	0.56	7.21	4.36	0.15	2.45	0.74	1.79	2.37	0.42	0.35	0.91	2.21
II	Cases	23	33	34	9	9	7	17	18	17	23	23	23
	Mean	+239.7	+187.0	+189.4	+241.3	+271.7	+22.9	+228	+88	0	+259	+164	−5
	σ	64.5	70.9	59.1	86.14	35.99	59.55	66.38	78.01	23.60	82.15	103.82	34.16
	Range	+303– +63	+345– +2	+285– +62	+352– +122	+328– +222	+164– −38	+320– +106	+301– −14	+42– −46	+382– +82	+372– +34	+116– −36
	Per cent	91	70	50	44	56	86	47	72	65	61	74	83
	c.r. 2:3	0.81	1.22	0.41	0.76	4.74	1.25	0	0.95	1.17	7.49	4.73	1.71
III	Cases	18	14	13	6	6	4	11	9	10	13	12	11
	Mean	+226.7	+161.9	+195.4	+225.7	+203.2	−0.75	+205	+67	−10	+144	+78	−20
	σ	79.64	100.6	65.5	6.63	54.44	32.14	209.13	73.52	35.51	50.62	50.46	32.17
	Range	+333– −13	+300– −13	+258– −8	+234– +217	+252– +92	+42– −48	+292– +92	+228– −33	+43– −53	+220– +50	+182– +6	+32– −57
	Per cent	72	57	92	50	83	50	100	78	70	69	67	55
	c.r. 1:3	1.19	5.30	2.60	2.56	2.16	2.28	1.43	2.76	0.49	6.19	2.78	2.62
	c.r. 3:4	0.05	1.62	1.11	1.20	1.51	1.57	1.84	3.80	0.53	1.60	0.99	0.11
IV	Cases	4	5	4	6	5	3	3	3	3	9	5	4
	Mean	+229.0	+85.6	+211.5	+176	+141.2	−33.3	+296	+136	+3	+128	+117	−21
	σ	113.73	128.0	17.84	128.96	111.80	34.66	45.6	9.76	42.35	94.63	111.44	16.72
	Range	+362– +79	+212– −121	+230– +187	+392– +4	+342- +3	+3- −80	+348– +237	+264– +22	+473– −54	+332– −2	+337– +42	−3– −48
	Per cent	50	80	50	67	60	33	66	33	33	55	80	50
	c.r. 1:4	1.66	2.63	3.58	1.66	2.63	3.58	0.55	1.24	0.39	8.56	1.54	2.66
	c.r. 2:4	1.42	3.37	2.58	1.42	3.37	2.58	2.77	3.56	0.13	11.07	1.17	1.89

* Results expressed as millivolts.

TABLE IV

rH

INCUBATION:		AEROBIC			AEROBIC + CASEIN			ANAEROBIC			ANAEROBIC + CASEIN		
Group	Hours:	1	3	24	1	3	24	1	3	24	1	3	24
I	Cases	3	6	6	4	4	4	3	3	3	4	4	4
	Mean	21.80	23.31	23.91	20.04	20.29	13.97	23.80	20.40	15.80	20.97	19.25	14.91
	σ	0.51	1.56	1.62	0.38	0.80	0.91	1.43	4.14	1.77	1.14	3.20	2.62
	Range	21.07–22.19	21.29–25.43	21.57–26.00	20.53–19.50	20.94–19.00	15.33–13.07	25.21–21.85	24.89–14.90	18.25–14.10	19.77–22.33	23.44–15.73	18.43–12.00
	Per cent	100	50	50	75	75	75	67	33	67	50	50	50
	c.r. 1:2	3.29	2.90	1.18	0.41	2.97	0.93	1.77	1.68	2.63	0.82	0.84	2.19
II	Cases	23	33	33	9	9	7	17	18	17	23	23	23
	Mean	23.14	21.74	23.27	20.40	21.82	14.80	22.40	17.00	13.50	21.44	18.10	12.64
	σ	2.24	2.24	1.91	3.62	1.75	2.99	2.25	2.69	0.74	2.59	3.40	1.20
	Range	25.83–17.36	26.18–15.57	26.70–19.27	14.83–25.03	19.47–24.75	21.67–12.25	26.01–18.53	24.23–12.91	15.08–12.25	25.35–15.25	25.19–13.63	16.87–11.59
	Per cent	74	70	73	33	67	86	53	72	59	70	70	83
	c.r. 2:3	0.82	1.20	0.61	0.47	3.26	2.40	2.00	1.25	1.38	6.66	4.49	1.95
III	Cases	18	14	13	6	6	4	10	9	10	13	12	11
	Mean	22.67	20.89	23.62	20.00	19.46	12.65	21.00	16.00	13.10	17.80	15.26	12.09
	σ	2.95	3.52	2.69	0.33	1.96	0.92	2.66	2.64	1.28	2.06	1.89	1.06
	Range	26.26–14.17	26.45–14.87	26.60–15.17	20.49–19.53	21.17–15.47	13.78–11.24	24.9–15.9	22.0–12.9	14.82–11.52	20.49–13.20	18.70–12.53	13.79–10.74
	Per cent	78	57	85	67	83	75	70	78	60	69	67	83
	c.r. 1:3	1.60	2.99	0.40	0.25	1.25	2.63	3.09	2.11	3.00	5.30	3.01	2.76
	c.r. 3:4	0.06	1.63	0.83	1.81	1.96	0.43	3.05	2.33	0	1.55	0.31	1.65
IV	Cases	4	5	4	6	5	3	3	3	3	9	5	4
	Mean	22.57	18.01	24.12	17.82	16.97	13.36	23.60	18.00	13.10	16.39	15.71	11.58
	σ	4.14	4.85	0.72	3.98	3.34	3.41	1.32	1.12	1.41	3.42	4.15	0.53
	Range	16.93–27.47	10.33–22.59	22.99–24.99	24.37–12.77	23.00–12.86	18.13–10.39	24.70–21.80	22.7–14.2	11.5–14.9	23.77–11.20	23.73–12.0	12.40–10.93
	Per cent	50	80	50	50	60	67	67	33	33	67	80	50
	c.r. 1:4	0.47	3.12	0.36	1.84	2.85	0.37	0.16	1.19	2.48	4.93	1.88	3.19
	c.r. 2:4	0.35	2.25	2.34	1.75	4.04	0.79	1.67	1.39	0.63	5.63	1.61	3.91

TABLE V

TABLE V

*Neutralization**

INCUBATION:		AEROBIC			AEROBIC + CASEIN			ANAEROBIC			ANAEROBIC + CASEIN		
Group	Hours:	1	3	24	1	3	24	1	3	24	1	3	24
I	Cases	4	6	6	4	4	4	3	3	3	4	4	4
	Mean	0.11	0.11	+0.19	0.87	0.77	1.31	0.19	0.34	0.59	0.86	1.25	1.26
	σ	0.07	0.10	0.24	0.22	0.19	0.29	0.09	0.95	0.18	0.29	0.42	0.11
	Range	0.20–0	+0.07–0.20	+0.70–0	0.50–1.05	0.50–0.99	0.99–1.76	0.08–0.30	0.20–0.56	0.40–0.84	0.56–1.26	0.81–1.81	1.15–1.45
	Per cent	50	83	83	75	50	50	33	100	33	50	50	75
	c.r. 1:2	2.07	1.12	1.84	0.98	0.48	1.72	0.04	0.04	0.81	3.13	0.01	10.39
II	Cases	22	32	31	9	9	7	18	18	17	23	23	23
	Mean	0.19	0.07	+0.33	0.77	0.82	0.94	0.17	0.36	0.52	1.28	1.26	2.39
	σ	0.19	0.17	0.20	0.18	0.33	0.68	0.11	0.17	0.17	0.48	0.35	0.67
	Range	0.65–0.03	0.72–+0.33	+0.02–+0.80	0.50–1.07	0.34–1.45	0 –1.61	0.05–0.45	0.14–0.77	0.33–0.88	0.57–2.93	0.70–2.00	1.32–3.63
	Per cent	68	59	84	67	67	71	56	67	71	78	65	70
	c.r. 2:3	1.00	2.11	1.24	1.13	2.10	0.83	0.65	0.06	0.39	0.87	3.06	1.10
III	Cases	19	12	12	6	6	4	12	11	11	12	13	11
	Mean	0.22	0.19	+0.17	0.88	1.09	1.43	0.16	0.39	0.53	1.20	1.57	2.56
	σ	0.16	0.24	0.62	0.27	0.33	0.46	0.12	0.28	0.19	0.34	0.45	0.73
	Range	+0.07–0.45	+0.15–0.63	+0.81–1.15	0.60–1.34	0.69–1.55	0.97–2.20	0.03–0.46	0.10–1.17	0.25–0.86	0.79–2.00	0.81–2.45	1.32–4.13
	Per cent	47	58	75	67	67	75	83	82	82	69	58	82
	c.r. 1:3	2.72	1.32	0.14	0.01	2.58	0.21	0.58	0.11	0.06	2.55	1.71	8.02
	c.r. 3:4	3.07	2.60	3.09	2.98	1.80	1.39	0.75	1.24	0.03	2.50	1.42	1.62
IV	Cases	4	6	4	6	5	3	3	3	3	9	5	4
	Mean	0.12	0.59	+0.58	1.62	1.53	3.74	0.13	0.31	0.54	1.70	1.84	2.18
	σ	0.05	0.48	0.15	0.78	0.67	3.28	0.06	0.05	0.07	0.80	0.49	0.45
	Range	0.05–0.20	0.09–1.49	+0.45–+0.81	0.74–2.56	0.82–2.40	1.23–8.38	0.05–0.21	0.26–0.38	0.45–0.63	0.82–3.25	1.55–2.58	1.77–2.87
	Per cent	50	67	50	33	40	67	33	67	33	67	80	75
	c.r. 1:4	0.06	3.20	4.78	3.00	3.22	1.55	1.09	0.07	0.58	3.78	2.53	5.14
	c.r. 2:4	2.42	3.49	5.51	3.53	2.98	1.78	1.16	0.18	0.44	2.08	3.40	0.90

* Results expressed as cc. of 0.01 NaOH per 1 cc. of saliva.
+ = cc. of 0.01 N H_2SO_4 per 1 cc. of saliva.

TABLE VI

*Formol titration**

INCUBATION:		AEROBIC			AEROBIC + CASEIN			ANAEROBIC			ANAEROBIC + CASEIN		
Group	Hours:	1	3	24	1	3	24	1	3	24	1	3	24
I	Cases	4	6	6	4	4	4	3	3	3	4	4	4
	Mean	0.51	0.86	2.02	0.99	1.42	5.83	0.34	0.66	1.88	1.03	1.44	5.65
	σ	0.13	0.24	0.52	0.04	0.17	1.67	0.05	0.08	0.64	0.36	0.43	2.33
	Range	0.36–0.69	0.58–1.24	1.23–2.70	0.94–1.04	1.30–1.70	4.80–8.72	0.29–0.40	0.55–0.75	1.18–4.31	0.50–1.46	0.98–2.12	2.80–9.27
	Per cent	50	33	50	50	75	75	67	33	33	50	50	50
	c.r. 1:2	5.62	4.79	2.51	4.28	3.14	2.38	4.94	7.32	1.37	2.93	2.80	3.03
II	Cases	21	33	33	9	9	7	18	18	17	18	23	23
	Mean	0.95	1.25	1.60	1.37	1.83	7.57	0.62	1.26	2.30	1.48	1.96	8.57
	σ	0.41	0.30	0.50	0.36	0.47	1.21	0.31	0.43	0.44	0.37	0.56	2.25
	Range	0.22–1.91	0.58–1.99	0.31–3.54	0.80–1.89	1.10–2.37	6.16–10.03	0.22–1.46	0.45–1.96	1.58–3.22	0.81–2.02	0.89–2.89	4.23–13.33
	Per cent	71	73	85	56	56	71	72	67	71	61	61	65
	c.r. 2:3	1.25	2.60	2.94	2.16	3.73	0.35	1.78	0.59	3.40	2.85	6.10	1.13
III	Cases	18	14	12	6	6	4	12	11	11	13	12	11
	Mean	1.07	1.58	2.36	1.84	2.68	7.86	0.75	1.34	2.85	1.72	2.76	9.35
	σ	0.47	0.66	1.24	0.66	0.66	1.94	0.27	0.58	0.67	0.29	0.51	2.87
	Range	0.32–2.09	0.25–3.08	0.59–5.50	1.15–2.71	2.01–3.64	5.97–11.10	0.38–1.41	0.53–2.78	1.64–4.32	1.19–2.25	1.82–3.48	5.07–15.04
	Per cent	72	72	67	67	67	75	75	82	73	85	67	73
	c.r. 1:3	6.24	5.06	1.15	4.24	6.02	2.04	6.94	5.22	2.87	4.52	6.73	3.38
	c.r. 3:4	3.13	0.11	2.67	2.59	1.72	1.56	2.45	2.97	1.43	2.60	1.05	0.03
IV	Cases	5	6	5	6	5	3	3	3	3	9	5	4
	Mean	1.82	1.60	3.40	2.73	3.39	9.71	0.55	0.92	2.46	2.16	3.23	9.37
	σ	0.67	0.45	0.88	0.94	1.08	1.93	0.13	0.15	0.49	0.65	1.29	1.61
	Range	1.16–3.10	1.17–2.50	2.14–4.62	1.26–4.19	2.05–4.83	7.40–12.11	0.40–0.72	0.74–1.13	1.99–3.14	1.30–3.72	2.37–5.97	7.13–11.02
	Per cent	80	83	60	67	40	67	33	33	66	79	80	50
	c.r. 1:4	5.68	4.24	4.11	6.16	5.34	3.44	3.09	3.10	1.49	5.31	3.83	3.37
	c.r. 2:4	3.71	2.49	5.95	4.62	4.11	2.19	0.87	3.40	0.63	4.26	2.87	1.14

* Results expressed as cc. of 0.01 N NaOH per cc. of saliva.

TABLE VII

*Indole**

INCUBATION:		AEROBIC			AEROBIC + CASEIN			ANAEROBIC			ANAEROBIC + CASEIN		
Group	Hours:	1	3	24	1	3	24	1	3	24	1	3	24
I	Cases	4	6	6	4	4	4	3	3	3	4	4	4
	Mean	0	0.23	1.92	0.21	0.73	25.10	0	0.27	3.48	0.01	0.36	18.98
	σ	0	0.54	1.58	0.26	1.08	13.81	0	0.27	1.39	0.02	0.21	11.84
	Range	0	0–0.40	0.74–5.20	0–0.66	0–2.60	11.40–48.00	0	0–0.63	2.00–5.38	0–0.05	0.14–0.65	3.50–33.40
	Per cent	100	100	83	75	75	75	100	67	33	75	50	50
	c.r. 1:2	6.88	2.27	0.25	1.42	0.05	0.59	3.77	4.48	3.36	5.73	4.63	0.58
II	Cases	22	33	33	9	9	7	17	17	17	23	23	23
	Mean	0.25	0.62	1.79	0.39	0.70	28.45	0.12	0.11	6.09	0.19	1.46	16.24
	σ	0.24	0.45	1.34	0.35	0.51	6.46	0.19	0.88	2.38	0.21	1.55	5.52
	Range	0–0.80	0–2.02	0.16–5.30	0.12–1.13	0.18–1.50	22.5–40.40	0–0.68	0–3.18	2.95–13.0	0–0.80	0.17–7.50	9.0–27.5
	Per cent	50	58	73	78	56	86	88	53	82	83	87	65
	c.r. 2:3	3.79	2.54	1.88	1.36	2.45	0.99	2.02	1.76	2.00	3.99	3.34	0.72
III	Cases	19	11	11	6	6	4	12	10	11	13	12	11
	Mean	0.75	1.38	2.96	0.64	3.16	33.38	0.25	1.85	7.48	0.63	3.07	17.15
	σ	0.80	1.39	2.82	0.52	3.07	11.93	0.26	1.74	2.66	0.55	2.09	4.57
	Range	0–2.43	0.06–5.0	0.10–9.50	0.13–1.35	0.10–8.95	21.0–45.6	0–0.75	0–6.00	4.10–11.20	0.03–1.70	0.53–6.60	9.70–25.0
	Per cent	84	82	82	67	83	50	75	70	55	69	58	55
	c.r. 1:3	5.91	3.42	1.36	2.28	2.39	1.16	4.73	3.84	4.55	5.81	6.24	0.35
	c.r. 3:4	0.55	0.60	3.54	2.26	1.85	1.41	3.86	1.84	0.44	3.17	2.29	2.42
IV	Cases	4	5	5	6	5	3	3	3	3	9	5	4
	Mean	0.62	1.60	8.30	3.34	7.38	46.00	0.05	0.92	7.97	2.14	6.29	21.98
	σ	0.50	0.65	4.10	3.94	6.19	16.08	0.07	0.67	2.11	1.95	4.10	4.47
	Range	0.16–1.40	0.50–2.50	4.0–15.5	0.13–11.4	1.13–19.0	24.0–62.0	0–0.15	0.16–1.78	6.0–10.9	0.17–6.60	2.70–14.10	16.40–28.80
	Per cent	75	60	60	83	60	67	67	33	67	67	80	50
	c.r. 1:4	3.17	5.03	4.37	2.63	3.12	2.23	1.50	1.90	3.69	4.59	4.23	0.61
	c.r. 2:4	1.87	4.36	4.67	2.48	3.14	2.23	1.56	0.54	1.73	4.19	3.45	3.00

* Results expressed as micrograms per cc. of saliva.

TABLE VIII

*Sulfides**

INCUBATION:		AEROBIC			AEROBIC + CASEIN			ANAEROBIC			ANAEROBIC + CASEIN		
Group	Hours:	1	3	24	1	3	24	1	3	24	1	3	24
I	Cases	4	6	6	4	4	4	3	3	3	4	4	4
	Mean	0.12	0.18	0.29	0.15	0.34	2.46	0.16	0.66	3.35	0.08	0.19	4.93
	σ	0.06	0.07	0.11	0.04	0.06	0.95	0.12	0.34	1.27	0.06	0.11	3.76
	Range	0.08–0.16	0.12–0.28	0.16–0.50	0.10–0.20	0.30–0.45	1.10–3.55	0–0.28	0.33–1.13	2.50–4.80	0–0.16	0–0.28	0.90–9.90
	Per cent	75	67	67	50	75	50	67	67	67	50	75	50
	c.r. 1:2	4.04	6.01	1.21	2.34	2.79	1.09	0.70	4.14	3.78	4.80	5.03	2.21
II	Cases	14	28	27	9	8	7	17	18	17	23	21	23
	Mean	0.23	0.46	0.23	0.20	0.23	3.45	0.50	1.65	6.09	0.26	0.80	8.61
	σ	0.08	0.33	0.25	0.06	0.12	3.00	0.71	1.33	2.37	0.20	0.75	5.53
	Range	0.06–0.36	0.13–1.46	0.01–1.10	0.10–0.30	0.10–0.40	0.84–10.60	0.05–2.80	0.19–4.68	1.85–12.0	0–0.93	0.16–3.60	2.50–27.50
	Per cent	71	86	89	67	63	86	94	67	82	87	95	78
	c.r. 2:3	2.90	1.74	1.12	3.76	11.86	1.37	0.05	2.05	1.98	3.10	1.26	1.09
III	Cases	11	10	11	6	6	4	11	10	11	13	12	11
	Mean	0.84	0.81	0.37	0.29	0.79	6.03	0.57	2.48	8.00	0.54	1.05	10.06
	σ	1.02	0.86	0.53	0.06	0.43	4.34	0.33	1.52	4.11	0.43	0.78	4.97
	Range	0.12–3.00	0.12–2.85	0–1.93	0.24–0.40	0.30–1.60	2.30–12.90	0.08–1.00	0.28–5.35	1.75–14.20	0.02–1.40	0.20–2.80	5.0–20.5
	Per cent	82	80	91	83	67	75	55	65	55	78	67	82
	c.r. 1:3	3.41	3.22	0.63	5.86	10.08	2.06	0.73	5.41	4.36	5.29	5.21	2.84
	c.r. 3:4	0.28	1.38	0.30	2.79	0.72	1.60	2.43	4.60	0.05	2.69	3.30	1.62
IV	Cases	2	2	4	6	5	3	1	1	3	2	5	4
	Mean	1.01	1.74	0.42	1.18	1.00	11.0	0.74	4.85	7.93	1.57	2.20	14.25
	σ	0.87	0.96	0.36	1.06	0.87	5.46	0	0	2.44	1.57	0.93	6.06
	Range	0.14–1.88	0.78–2.70	0.10–1.04	0.16–2.60	0.22–2.70	6.00–18.60	0.74	4.85	4.50–9.90	0.23–5.45	1.25–3.60	8.10–21.80
	Per cent	100	100	75	67	80	67	100	100	67	89	80	75
	c.r. 1:4	1.51	2.41	0.90	3.24	2.26	3.24	1.05	36.03	3.50	3.90	6.36	3.36
	c.r. 2:4	1.33	1.97	1.29	3.07	2.63	2.76	20.12	11.03	1.50	3.49	3.94	2.27

* Results expressed as micrograms per cc. of saliva.

TABLE IX

*Tryptophane**

INCUBATION:		AEROBIC			AEROBIC + CASEIN			ANAEROBIC			ANAEROBIC + CASEIN		
Group	Hours:	1	3	24	1	3	24	1	3	24	1	3	24
I	Cases	3	6	6	4	4	4	3	3	3	4	4	4
	Mean	27.7	23.0	15.7	87.5	71.3	51.0	21	9	6	63.5	102.5	29.7
	σ	14.29	13.71	12.58	22.87	38.87	13.21	11.11	1.89	0.82	30.34	31.70	7.09
	Range	10–45	10–49	5–40	51–113	25–119	31–66	10–36	5–10	5–7	24–93	62–140	18–36
	Per cent	33	83	83	50	50	50	67	67	33	75	50	75
	c.r. 1:2	0.80	1.00	0.14	0.07	0.42	3.07	0.02	2.64	0.86	1.26	1.29	1.08
II	Cases	15	31	31	9	9	7	17	18	17	22	23	23
	Mean	22.1	18.8	15.2	79.8	64.4	27.6	21	12	5	79.5	83.4	34.1
	σ	8.83	7.78	7.78	27.88	24.46	20.46	5.26	4.68	3.33	32.21	35.10	21.11
	Range	9–42	4–35	0–32	15–113	25–106	7–70	8–29	3–21	0–12	16–136	21–140	8–86
	Per cent	60	52	71	67	67	86	71	72	77	68	70	57
	c.r. 2:3	1.59	0.22	1.42	1.10	5.49	4.84	0.13	2.09	0.98	2.78	0.50	0
III	Cases	13	10	12	6	6	4	12	10	11	13	12	11
	Mean	18.4	18.4	13.3	89.3	106.0	59.5	21	16	5	101.8	81.7	34.1
	σ	8.82	6.23	4.66	18.12	16.04	8.76	9.94	8.17	2.60	33.11	27.87	31.27
	Range	6–35	10–28	5–20	56–109	82–127	48–72	4–36	5–28	0–8	21–160	29–137	0–102
	Per cent	62	60	75	50	83	50	65	60	64	85	75	73
	c.r. 1:3	1.32	1.05	0.63	0.17	2.19	1.38	0.11	3.57	2.01	2.84	1.53	0.61
	c.r. 3:4	1.95	5.63	1.27	0.35	0.37	5.67	1.33	0.60	1.40	0.50	1.19	0.64
IV	Cases	3	4	4	6	5	3	3	3	3	9	5	4
	Mean	36.7	5.0	20.3	94.0	113.8	37.0	18	17	2	95.8	102.8	28.38
	σ	19.36	4.36	13.97	40.96	61.26	4.24	3.85	1.89	3.30	41.65	49.88	16.21
	Range	17–63	1–13	8–44	58–175	64–225	31–40	14–23	16–20	0–7	35–188	51–190	11–53
	Per cent	67	75	75	83	80	67	33	67	67	78	60	50
	c.r. 1:4	0.78	3.80	0.69	0.43	1.66	2.53	0.53	7.11	2.29	2.09	0.01	0.21
	c.r. 2:4	1.55	6.55	0.92	1.01	2.30	1.57	1.58	4.91	3.21	1.48	0.99	0.84

* Results expressed as micrograms per cc. of saliva.

Neutralization and free carboxyl groups: Before a formol titration can be made, it is necessary to adjust the pH of the saliva to the end point of phenolphthalein. This process gives a rough estimate of the neutralizing capacity of the saliva. Insofar as these data were available, they were analyzed for significance. As these values are closely correlated with the pH of the saliva, no close correlation

TABLE X

pO

INCUBATION:		AEROBIC			AEROBIC + CASEIN			ANAEROBIC			ANAEROBIC + CASEIN		
Group	Hours:	1	3	24	1	3	24	1	3	24	1	3	24
I	Cases	4	6	6	4	4	4	3	3	3	4	4	4
	Mean	2	3.5	6.3	2.3	4.3	7.8	2	3	6.7	1.3	2.8	7.8
	σ	0.71	0.5	0.94	0.43	0.83	1.64	0	0.82	1.25	0.43	0.83	0.82
	Range	1–3	3–4	5–8	2–3	3–5	5–9	2–2	2–4	5–8	1–2	2–4	7–9
	Per cent	50	100	67	75	75	75	100	33	100	75	75	75
	c.r. 1:2	5.49	8.85	2.93	1.91	2.49	1.17	1.99	2.65	2.13	9.39	4.98	2.65
II	Cases	17	31	31	9	9	7	17	18	17	23	23	23
	Mean	3.7	5.4	7.2	3.1	5.2	8.6	2.2	4.2	8.0	3.0	4.6	8.7
	σ	0.71	1.18	0.67	1.73	0.92	1.05	0.78	1.21	1.31	0.62	1.31	0.76
	Range	3–5	3–8	4–8	1–6	4–7	6–9	1–4	2–6	6–9	2–4	3–7	6–9
	Per cent	88	58	55	56	67	86	76	44	82	61	65	91
	c.r. 2:3	5.90	5.30	3.18	1.16	1.27	1.49	4.23	3.63	1.52	3.31	5.86	2.06
III	Cases	14	12	16	6	6	4	12	11	11	13	12	11
	Mean	5.1	7.0	7.7	3.8	6.2	9	3.2	5.5	7.6	4.1	6.3	8.9
	σ	1.19	1.35	0.58	1.57	2.37	0	1.14	1.37	0.64	1.54	1.11	0.29
	Range	2–7	4–8	6–8	2–7	3–9	9–9	2–6	4–8	6–8	2–8	5–9	8–9
	Per cent	86	83	75	67	50	100	92	55	91	69	67	91
	c.r. 1:3	8.88	11.15	4.50	3.14	2.45	1.96	5.02	5.01	1.59	8.23	9.10	3.53
	c.r. 3:4	0.40	0	0.08	4.03	2.92	0	2.59	0.02	2.63	7.45	9.42	1.47
IV	Cases	4	4	6	6	5	3	3	3	3	9	5	4
	Mean	5	7	7.7	7.0	8.4	9	2.3	5.3	9	7.3	8.8	9.0
	σ	0.70	1.00	0.76	2.08	0.80	0	0.47	0.94	0	1.33	0.40	0
	Range	4–6	6–8	6–8	4–9	7–9	9–9	2–3	4–6	9–9	5–9	8–9	9–9
	Per cent	50	100	83	83	80	100	67	67	100	67	80	100
	c.r. 1:4	4.50	3.97	1.79	7.30	9.86	1.96	1.46	3.91	2.61	16.93	17.29	3.87
	c.r. 2:4	7.70	8.38	3.68	5.19	9.16	1.49	0.63	2.37	0	13.00	18.06	3.19

with the clinical findings was expected. This was the case and only scattered critical ratios of 3 or more were obtained. When the results of the formol titration were analyzed, it was found that they were highly significant at the 1 and 3 hour intervals with all methods of incubation. As would be expected from our method of dividing the cases into 4 groups on the basis of clinical and radiographic examination, the differences observed from group to group were not always significant; but when normals were compared to diseased individuals, significance was obtained. There is very little choice in regard to the method of incubation, as the degree of significance is about the same with all methods.

In general, the hydrolysis progressed rapidly with all samples, but the saliva from the pathological cases progressed much more rapidly early in the incubation than did the normal. The results with the 24 hour samples were not usually significant because with this elapsed time all of the reactions had approached completion.

Indole: The presence of indole as the result of decarboxylation, deaminization and oxidation-reduction should be an accurate criterion of putrefaction beyond the hydrolysis stage. Here again we observe that indole is formed quite rapidly in all cases, but much more rapidly in the pathological specimens. At the 24 hour interval the differences between the pathological and normal saliva is small. This presumably is because at this time interval all of the reactions have approached completion. Differences between the groups are, in general, significant at the 1 and 3 hour intervals, particularly when the samples are incubated anaerobically with added casein.

Sulfides: The production of sulfides would also be a measure of the putrefaction process after hydrolysis has taken place. Here again we find the same trend as with indole. A very peculiar phenomenon was found, wherein many cases, after an initial rise in sulfides, showed a distinct decrease at the 24 hour interval. This was not observed when the samples were incubated anaerobically. It was found that this was due to an oxidation of the sulfide to the sulfate and was usually reflected in the oxidation-reduction potential. The differences between the groups were, in general, significant. The method of incubation which showed the greatest differences was the anaerobic plus casein at the 1 and 3 hour intervals.

Tryptophane: The presence of free tryptophane should be a criterion of the degree of hydrolysis, provided the tryptophane was not rapidly degraded. From the results obrained., it is obvious that the amino acid was broken down almost as fast as it was formed. This is further shown by the rapid increase in indole concentration which could only be produced by the degradation of tryptophane. In view of this rapid degradation of the amino acid, it is obvious that the rate of hydrolysis of the protrin is much more rapid than is indicated by the formol titration.

Odor concentration: The measurement of odor concentration is based on the olfactory organ and hence cannot be purely objective; however, by means of the osmoscope and with practice, checks may be obtained with remarkable accuracy, providing the odors are relatively dilute. Any reading up to a pO value of 6 is quite accurate, but readings above pO 6 are very inaccurate and only rough guesses. For this reason, the analyses at the 1 and 3 hour intervals are quite accurate, but those on the 24 hour specimens are of little value. The results were similar to those previously obtained. In all cases, the odor concentration increased rapidly under all forms of incubation and the pathological saliva putrefied much more rapidly than did the normal saliva. The results when saliva was incubated anaerobically with casein were particularly significant.

Other observations not indicated by the statistics: There were many observations not evidenced by the statistics which may be of value. It was noted that the state of cleanliness of the mouth did not alter the rate of putrefaction of the saliva for an appreciable period of time. A complete prophylaxis would cause a slight

decrease in the rate of putrefaction for a period of 2 or 3 days. This slight decrease was not ordinarily of sufficient magnitude to cause the saliva of a member of Group 4 to react like that of Group 1. This may indicate that the number of bacteria present in the surface filth is not the determining factor in the rate of putrefaction.

It was noted that an infected third molar operculum would cause the saliva from an otherwise normal mouth to putrefy very rapidly. The rate of putrefaction in these cases was often similar to that of saliva from individuals in Group 4.

A few case s were observed shortly after the use of penecillin lozenges. These cases had a very low rate of putrefaction.

A severe "cold" would in some cases cause the saliva to putrefy more rapidly than saliva from the same source if gathered when the patient did not have the "cold."

It was noted that the presence of sugar in the saliva, even in very small quantities, would materially decrease the rate of putrefaction. This may be an important factor in explaining the clinical observation that caries and periodontal disease are not usually active simultaneously.

Although the rate of putrefaction seemed to correlate very well with the periodontal conditions of the mouth, there was no evidence to indicate whether the rate of putrefaction was a cause or an effect of this condition.

CONCLUSIONS

There is a marked difference in the rate of putrefaction of the saliva from individuals with and without periodontal disturbance. The differences in rate of hydrolysis production of indole, sulfides and odors are particularly significant when saliva is incubated under anaerobic conditions and in the presence of added substrate. The differences in pH, Ec, EH, rH and neutralizing capacity are of little significance.

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BLOOD STUDIES IN PERIODONTOCLASIA. II[1]

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One of the procedures for studying the etiology of periodontoclasia is to analyze blood for constituents which are known to undergo changes in content in disease. From time to time reports have appeared indicating higher than normal values for total calcium (1–7), total cholesterol (1, 2, 4, 8), uric acid (3, 5, 8, 9), sugar (1, 2, 4, 5, 6) and local phosphatase (10) in various percentages of periodontoclasia cases. Local phosphatase refers to phosphatase in gingival blood. Lower than normal values have been reported for vitamin C (11–14), erythrocyte count (15) and CO_2—combining power (4, 16).

Recently Tenenbaum and Karshan (17) reported on the blood chemistry of 70 patients with advanced periodontoclasia, and of 22 persons who were free from this disease. On the basis of the percentage of cases in each group which gave values outside of the normal ranges as reported in the literature, they concluded that their results suggest that some systemic condition was one of the contributing factors in periodontoclasia in many of the subjects studied. This study is referred to as the first series when further mention of it is made in this report.

In an extension of this investigation, 67 periodontoclasia cases, 15 to 58 years of age, and 32 control cases 18 to 54 years of age have been studied. In this series, which is referred to as the second series, the degree of periodontoclasia ranged from incipient to advanced. In addition to blood analyses, determinations were made of basal metabolism, and urinary tests were conducted to ascertain the possible presence of subclinical deficiencies of thiamin, riboflavin and nicotinic acid.

METHODS FOR BLOOD AND URINE ANALYSES

Blood determinations were made on a fasting basis. Approximately 40 cc. of blood were drawn from the arm at about 9:30 a.m. and the following determinations carried out:

A. In serum. 1. Total calcium.[2] Kramer and Tisdall (18), as modified by Clark and Collip (19). The precipitate of calcium oxalate was washed twice with ammonium hydroxide, the second time without disturbing the precipitate,

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[2] Represent analyses made also in the first series. In that series total cholesterol and CO_2-combining power were determined in oxalated blood, and in the vitamin C determination KCN was not added as in the Prunty and Vass procedure.

but with thorough washing of the wall of the centrifuge tube. Calcium-ion concentration was read from nomogram on basis of total calcium and total protein. McLean and Hastings (20). 2. Inorganic phosphate[2] and phosphatase.[2] Bodansky (21, 22). 3. Total protein[2], albumin. Greenberg (23). Globulin was estimated as the difference between total protein and albumin. 4. Total cholesterol. Sperry (24). 5. Free cholesterol. Sperry (25). 6. Vitamin A and carotene. Adaptation of method of May (26). 7. Sodium. Salit (27). 8. Chloride. Wilson and Ball (28). 9. Cholesterol-cephalin flocculation. Hanger (29).

B. In whole blood. 10. Sugar.[2] Benedict (30). 11. Non-protein nitrogen. Kock and McMeekin (31). 12. Urea nitrogen. Looney (32). 13. Uric acid.[2] Folin (33). 14. Hemoglobin. Osgood and Haskins (34). 15. Erythrocyte sedimentation rate[2]. Haskins, Trottmann, Osgood and Mathieu (35). In this procedure, values above 5 mm. which represent the difference between the 15 minute and 45 minute readings, are considered abnormal. 16. Erythrocyte, leukocyte and differential counts. Todd and Sanford (36).

C. In oxalated plasma. 17. Vitamin C[2]. Prunty and Vass (37).

The urinary tests for subclinical deficiencies of thiamin, riboflavin and nicotinic acid are based on the hypothesis of Holt and Najjar (38) that the presence of thiamin, riboflavin and N^1-methylnicotinamide in a specimen of urine collected at a definite time indicates that the body has a surplus available for excretion. On the other hand, if excretion of these substances during the specified period falls to zero, it indicates that no suprlus is available for excretion, and that such an individual is potentially deficient in these vitamins. In these tests, the subject eats the regular evening meal at about 7 p.m. At 7 a.m. the next morning, urine is voided and discarded, and immediately thereafter a glass of water is ingested. At 8 a.m. a specimen of urine is collected for the vitamin determinations. It has been found that following a meal there is a marked increase in thiamin, riboflavin and N^1-methylnicotinamide excretion. After about 8 hours, the rate of excretion falls to almost constant levels which are determined by the stores of the vitamins in the body. Measurement of the excretion during an arbitrary period, as for example, during the thirteenth hour after a meal, serves as a guide to the body stores of vitamin.

The methods used to determine the presence of thiamin, riboflavin and N^1-methylnicotinamide in the urine were as follows: Thiamin. Adaptation of Fluorometric method of Hennessy and Cerecedo (39). Riboflavin. Najjar (40). N^1-methylnicotinamide. Najjar (41). As a check on this test, many of the specimens were tested for N^1-methylnicotinamide by the method of Huff, Perlzweig and Tilden (42) which became available while this investigation was in progress.

In order to determine the approximate amounts of thiamin, riboflavin and N^1-methylnicotinamide in the fasting hour excretion samples, the fluorescence of the specimens was compared with standards in a fluorocomparator. These standards were aqueous solutions containing 0.5 to 1 gamma of thiamin per 1 cc.; 1 to 3 gammas of riboflavin per 5 cc.; 50 to 100 gammas of N^1-methylnicotinamide chloride per 25 cc.

To evaluate the significance of the blood chemistry determinations, and basal metabolic rates, the differences between the means of the control and periodontoclasia groups were divided by the standard errors of the differences. When there were no significant differences between the means of the control groups of the

TABLE I

Blood values for which the means of the control and periodontoclasia groups show significant differences

	MEAN OF CONTROL GROUP	MEAN OF PERIO. GROUP	DIFFERENCE BETWEEN MEANS (D)	$\frac{D}{\sigma D}$
First and second series combined: 54 control cases, 137 perio. cases.				
Phosphatase Units/100 cc. serum	2.5	2.9	0.4	3.1*
First and second series combined: 38 control cases, 105 perio. cases.				
Erythrocyte sedimentation rate, Mm/15 minutes	2.8	6.1	3.3	5.0
First series: 22 control cases, 70 perio. cases.				
Total protein† (gm./100 cc. serum)	6.16	6.51	0.35	3.8
Sugar (mg./100 cc. blood)	77	92	15	3.9
Calcium (mg./100 cc. serum)	10.0	10.6	0.6	3.7
Second series: 32 control cases, 67 perio. cases.				
Total protein† (gm./100 cc. serum)	6.65	6.91	0.26	2.7
Globulin (gm./100 cc. serum)	1.98	2.22	0.24	2.5
Leukocyte count (No./cmm.)	6483	7641	1158	2.7
Basal metabolism (Cal./sq.m./1 hr.)	−8.6	−2.4	−6.2	2.9

* A value of 2.5 or more was considered significant.

† Different methods were used in the first and second series to determine total protein. This accounts for the higher values in the second series than in the first.

first and second series, and between the means of the periodontoclasia groups of the 2 series, both series were treated as a single one. Otherwise each series[3] was treated separately.

[3] Although the first series has already been reported, it is presented here because the data were not previously treated in this way.

RESULTS AND CONCLUSIONS

Blood values for which the means of the control and periodontoclasia groups show significant differences are given in Table I. None of the other constituents gave significant differences between the means of the 2 groups. However, in several instances where there were no significant differences between the means of the control and periodontoclasia groups, it is worth comparing the percentage of cases in each group which gave values outside the normal ranges. This is shown in Table II.

In the urine analyses, thiamin, riboflavin and N^1-methylnicotinamide were found in all of the 1-hour excretion samples. Therefore, these tests did not

TABLE II

Deviations from the normal ranges for some blood constituents

BLOOD CONSTITUENT	PERCENTAGE OF CASES IN CONTROL GROUP WITH VALUES OUTSIDE NORMAL RANGE		PERCENTAGE OF CASES IN PERIODONTOCLASIA GROUP WITH VALUES OUTSIDE NORMAL RANGE	
	Above normal range	Below normal range	Above normal range	Below normal range
First series: 22 control cases, 70 perio. cases.				
Vitamin C	0	13	0	33
Cholesterol*			14	5
Second series: 32 control cases, 67 perio. cases.				
Sugar	3	0	12	1
Erythrocytes	3	3	3	21
Lymphocytes	13	0	24	1

* In the case of cholesterol, the periodontoclasia group was compared with the control group because of the differences in ranges reported as normal.

reveal a subclinical deficiency of thiamin, riboflavin or nicotinic acid in any of the subjects. Furthermore, when the approximate amounts of these factors in the control and periodontoclasia groups were compared, no appreciable differences were noted between the groups.

The results of the blood analyses suggest, as previously stated (first series) that some systemic condition operated as one of the contributing factors in the periodontoclasia in some of the subjects studied. However, the nature of a systemic condition that may be involved cannot be definitely stated on the basis of the available data. Since a number of different conditions are responsible for deviations from the normal for each of the blood characteristics studied, the difficulty involved in interpreting the findings becomes obvious. It is quite possible in some instances, as in the cases of phosphatase content, erythrocyte

sedimentation rate, and erythrocyte and leukocyte counts that the relation may be one of effect rather than of cause.

In the periodontoclasia group (second series) there were 3 cases under the age of 20. Thirty-five out of 37 patients in this group between the ages of 15 to 33 years were females; whereas after 33 years of age, the patients were almost equally divided between the 2 sexes. This suggests, as reported previously, (first series) that periodontoclasia when it occurs at an early age, is most prevalent among females. In 18 of the 37 periodontoclasia patients between the ages of 15 and 33 years, it is not possible to account for their condition on the basis of local conditions such as (a) calculus accumulations, (b) malposed teeth and, (c) tooth loss with shifting resulting in abnormal occlussal relationship, loss of contact points and food impaction areas. One or more of these conditions are commonly assumed to be etiologically related to periodontoclasia. One or more of these local conditions existed in all of the cases above the age of 33 years.

SUMMARY

Blood was analyzed and basal metabolism tests performed in 67 patients with incipient to advanced periodontoclasia, and in 32 control cases who were free from this disease (second series). Urine was analyzed to test for a possible subclinical deficiency of thiamin, riboflavin and nicotinic acid. In a previous study, 2 of the authors (B. T. and M. K.) reported on the blood chemistry of 70 patients with advanced periodontoclasia, and of 22 control cases (first series). Combining the results of both studies, significant differences between the means of the control and periodontoclasia groups were found for serum phosphatase and erythrocyte sedimentation rate. In the second series significant differences between the means of the 2 groups were found for total serum protein, serum globulin, leukocyte count and basal metabolism. In the first series significant differences between the means of the groups were found for total serum protein, serum calcium and sugar. On the basis of the urinary tests, there was no evidence of a subclinical deficiency of thiamin, riboflavin or nicotinic acid in any of the subjects in either group.

We are indebted to Prof. John W. Fertig of the School of Public Health, Columbia University for the statistical anslysis.

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MYOBLASTOMA[1]

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With the exception of Weber (1), who in 1854 reported a tumor of the tongue with microscopic features suggestive of the myoblastoma, no reference is found to this tumor previous to 1926. In that year Abrikossoff (2) described a peculiar group of tumors which he believed arose from primitive myoblasts, following muscle injury. Until 1934 when Klemperer (3) reviewed the literature and reported 6 cases, the tumor was considered relatively rare. Gray and Gruenfeld (4) in 1937 tabulated the cases in the literature and reported 5 tumors answering the description of myoblastoma. The clinical and histologic features of the tumors in their group were essentially as described by other authors. In 1939 Frasdorf (5) reported bilateral myoblastomata in the pharynx in a 68 year old man, from which he concluded that a dysontogenic basis for their histogenesis might be considered. Grya and Gruenfeld suggested that the tumor cells arose from muscle fibers undergoing microbiotic changes. Meyer (6), on the other hand, felt that the neoplasm resulted from an abnormal differentiation of the mesenchymal cell which would ordinarily produce connective tissue. A number of malignant tumors have been reported as myoblastomas; however, a closer examination of some of these cases suggests a diagnosis of rhabdomyosarcoma. The files of the Registry of Dental and Oral Pathology at the present time contain 17 myoblastomas in the oral regions. These cases have been tabulated, examined, and will be discussed in this report.

REPORT OF CASES

Case I. A.I.P. Acc. 164784. A 25 year old white soldier complained of a swelling on the right side of the lower lip. Examination revealed a lesion approximately 1.5 cm. in diameter at the vermilion border on the middle portion of the right side of the upper lip. The duration of this lesion is unknown. At no time did the patient complain of pain. The tumor was firmly attached to the deeper structures of the lip, although it did not protrude into the oral cavity proper. He stated that he did not smoke, nor did he recall any trauma to that side of the mouth. There was no regional adenopathy. The tumor was removed by surgical excision with no untoward results.

Case II. A.I.P. Acc. 62494. A 52 year old female upon routine dental examination was found to have a tumor of the tongue which was diagnosed clinically as a papilloma. The tumor was located on the left margin of the tongue near the base. She had no symptoms at any time and was not aware of the presence of the lesion. It was removed under local anesthesia and the wound healed uneventfully. The patient was seen 1 year later and was in excellent physical condition. There was no recurrence of the lesion.

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Case III. A.I.P. Acc. 65655. A 2 weeks old female infant when seen had several irregular masses present on both the maxillary and mandibular mucosa in the anterior portion of the mouth (*fig. 3*). There was no history of injury, the parents stating that they believed the lesions had been present since birth. There was no evidence of tumor elsewhere on the body. The family history was noncontributory.

Case IV. A.I.P. Acc. 164307. A 35 year old white soldier was seen in the Ear, Nose, and Throat Clinic because of some difficulty of speech. Examination revealed a tumor approximately ¾ inch in diameter on the under surface of the anterior third of the tongue. This lesion had been present for about 6 months and was at no time painful. Some difficulty was experienced in manipulation of the tongue.

Case V. A.I.P. Acc. 164875. A 36 year old white soldier had a tumor nodule on the under surface of the tongue on the right side. The lesion had been present for 12 months and had occasionally been painful because of superficial ulceration. There was no regional

Fig. 1. Pseudo-epitheliomatous hyperplasia of epithelium covering tumor. This dyskeratotic activity has been often mistaken for squamous cell carcinoma. Note tumor cells at (A).

adenopathy and the remaining history was essentially negative. The tumor was removed under local anesthesia and repair was uneventful.

MICROSCOPIC FEATURES

The microscopic features of the 17 tumors being presented are essentially manifest in the 5 cases to be discussed in detail. In all instances the tumors were composed of large granular acidophilic cells, polyhedral in shape, ranging between 20 to 60 micra (*fig. 2*). The granules were essentially neutrophilic and in all instances were negative with Sudan stains. This is important inasmuch as these tumors are often mistaken for xanthomata. The nuclei were fairly uniform, although on occasion some minor discrepancy in staining characteristics was noted (*fig. 4*). The tumor cells were arranged in collections, these groups being surrounded by a connective tissue membrane, which, in some instances, was moderately collagenous (*fig. 6*). It should be stated that the myoblastic

cells were uniformly devoid of vacuoles. Some authors have suggested that glycogen granules may be present but appropriate stains failed to demonstrate this substance. In certain cell collections both longitudinal and cross striations

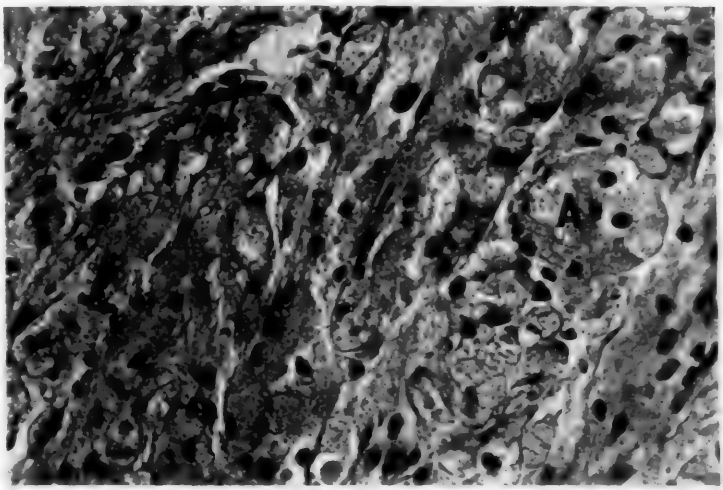

Fig. 2. The granularity of the myoblastic cells is clearly evident at (A). There is a slight variation in nuclear size. Mitoses are not evident. Note the cross-striations.

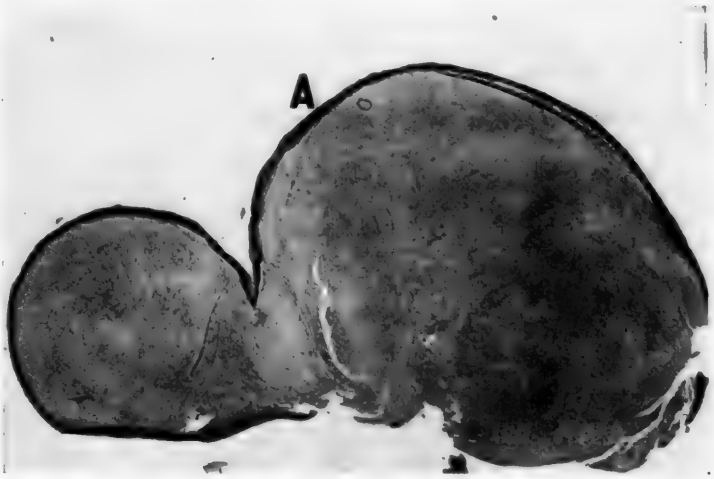

Fig. 3. Intra-oral lesion in child. The covering epithelium (A) is atrophic.

were markedly apparent (*fig. 2*), even to the extent of simulating fibrils. In poorly fixed tissue the granulation of the cell cytoplasm appeared excessive, a condition which is apparently overcome in freshly fixed and properly prepared material. An important finding was the tendency of the overlying epidermis, in

the case of certain tumors, to show considerable hyperplastic change (*fig. 5*). In some instances this approached a pseudoepitheliomatous hyperplasia which is

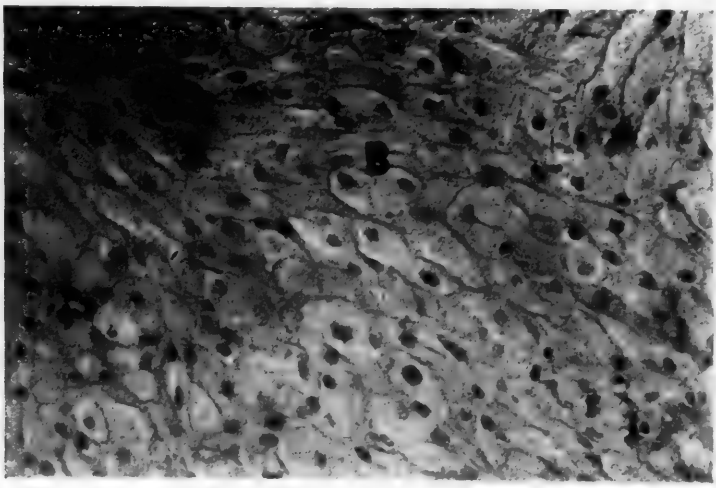

Fig. 4. Higher power of *fig. 3*. The granularity of the cells is striking. Note binucleated cell at (B).

Fig. 5. Hyperplastic activity of covering epithelium. The tumor (C) completely fills corium. There is no line of demarcation between the tumor and adjacent normal tissue. Note normal muscle tissue at (C).

occasionally mistaken for squamous cell carcinoma (*fig. 1*). It is questionable if such a change in these tumors is truly representative of a malignant process. One might suppose that the reaction of the epithelium is the result of the expansive growth of the underlying tumor. Case number III was a noteworthy exception.

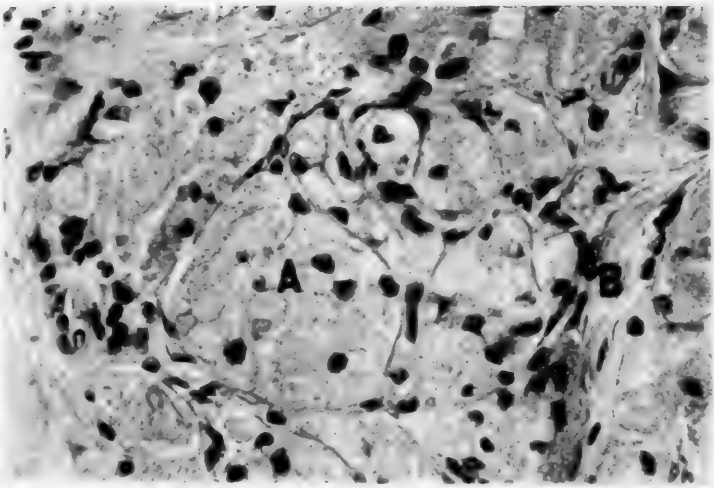

Fig. 6. The grouping of tumor cells is shown at (A). There is a wide band of collagenous fibrous tissue at (B). Note the thin connective tissue septa outlining the clumps of tumor cells.

TABLE I

AGE	RACE	SEX	DURATION	LOCATION	SYMPTOMS	SIZE	EPITHELIUM
2 wks	W	F	Since birth	Upper & lower gingivae	None	1.1 x 6 mm	
1	C	M	Since birth	Upper lip	None	Small	Ulceration
24	W	M	1 yr	Tip of tongue	None	Small	
25	W	M	?	Upper lip	None	Small	Hyperplasia
27	C	M	1 yr	Tongue	Pain	Small	
27	W	M	7 mo	Tongue	None	Small	Ulceration
28	C	M	9 wks	Tongue	Pain	Small	Hyperplasia
29	W	M	?	Tongue	None	3 x 4 mm	Hyperplasia
30	W	M	12 yrs	Tongue	None	7 x 8 mm	
34	W	M	4 mo	Tongue	None	6 mm	Hyperplasia Acanthosis
35	W	M	6 mo	Tongue	None	Small	Acanthosis
36	W	M	12 mo	Tongue	None	Small	Ulceration Hyperplasia
38	W	M	8 mo	Bridge of nose	None	Small	Ulceration
38	C	M	1 yr	Tongue	None	Small	
38	W	M	4 mo	Tongue	Pain	Small	Hyperplasia
39	W	M	8 mo	Tongue	None	Small	Acanthosis
52	W	F	?	Tongue	None	Small pea	Hyperplasia

In this instance the covering epithelium was atrophic (*fig. 3*) with focal areas of hyperplasia. Mitotic figures were not a common finding, although several investigators have reported their presence (7, 8).

Although, as stated previously, in most instances the tumor cells appeared to be arranged in clumps and surrounded by a connective tissue septum, this was not

a universal finding. Masson's trichrome stain failed to demonstrate these septa in certain areas, indicating direct apposition of the cells.

CLINICAL AND STATISTICAL DATA

Of the 17 cases being reported, 13, or 76.5%, affected the tongue, 1, or 5.9%, the skin of the cheek, 2, or 11.7%, the lip, and 1, or 5.9%, the mandibular and maxillary mucosa. Thus, it may be seen that the majority of those occurring in the oral regions were located on the tongue. This is in keeping with the reported

TABLE II

	CRANE AND TREMBLAY SUMMARY	CASES IN THIS REPORT
Tongue	61	13
Subcutis	17	
Skin	17	1
Muscle	13	
Maxilla	11	
Breast	8	
Larynx and vocal chord	8	
Mandible	6	
Lip	4	2
Ear	3	
Trachea and bronchi	3	
Alveolar process	2	1
Other	9	
Total	162	17

TABLE III

Average Age	40.2 years
Race	13 white (76.5%)
	4 colored (23.5%)
Sex	15 males (88.3%)
	2 females (11.7%)
Average Duration	12.2 months

figures of Crane and Tremblay (9) (see Table II). The tumors occur at any age and in our group appear to show a predilection for males (15, or 88.3%), although other authors have reported about equal frequency among males and females. The average period of duration was 12.2 months (Table III). This figure must be accepted as questionable, inasmuch as it is based entirely on the recollection of the patient. These data are summarized in Table I. Symptoms of any type were rare. In but 2 instances was pain an attending feature and in both cases could be attributed to the superficial position of the tumor where it was easily traumatized. In general, the tumors were small, seldom reaching a size greater than 1.5 cm. in diameter, cases IV and V being noteworthy exceptions. In 14

of the 17 cases it was possible to examine the overlying epithelium. In every instance hyperplasia was noted. This varied from a simple focal irregularity and elongation of the rete pegs to a severe pseudoepitheliomatous hyperplasia. In no instances was the epithelium normal. It is important to note that although this group of lesions all occurred in or about the mouth, the clinical features and distribution are essentially similar to previously published reports.

COMMENT

No unusual histologic features or clinical manifestations are apparent in this group of tumors. Considerable speculation has persisted as to the etiology of the myoblastoma. Abriskossoff, in his original report, suggested that their origin was associated with a degenerative process of muscle cells following trauma, but in a later paper suggested that certain of the lesions might have been associated with embryonic muscle cells on a dysontogenic basis. The similarity of the myoblastic cell to regenerating muscle cell is striking. Both Wolbach (10) and Godlewski (11) described the fibrillar arrangement of the granules in these cells and suggested that the mature myofibrils might be formed as a result of the consolidation of these granules. Gray and Gruenfeld are of the opinion that the lesions are essentially the result of degeneration of striated muscle cells, an opinion shared by Ewing (12). They, however, refer to the reported cases of Klinge (13) which occurred in locations devoid of striated muscle tissue, and feel that the regenerative theory of origin could not be applied to these tumors. Klinge explained the presence of these tumors according to Cohnheim's tumor theory, suggesting a dysontogenic background. Such a belief would, of necessity, lead one to suspect that the tumors reported in such areas are not oncologically identical with the myoblastoma. Their histologic similarity, however, is quite real and somewhat difficult to explain. Certainly there is a remarkable degree of consistency in the histologic features of tumors encountered in all locations. In a case recently examined in the Registry Office myoblastic-like changes were noted in a lip specimen which was removed following radium implantation. The original lesion was a basal cell carcinoma. In this case there was extensive degeneration of the muscle cells, strongly suggesting features of pseudomyoblastic activity on a regenerative basis. We are inclined to place considerable stress on the theory of regenerative myoblastic activity as an etiologic possibility. In several instances we have noted that following trauma the degenerating muscle cells assume a granular cytoplasm which, as the process begins to show regeneration, begins to consolidate, producing a fibrillar matrix. It seems possible that an initial degeneration followed by repair might, to some degree, explain these cytoplasmic changes. At one time the term "congenital epulis of the new-born" was used to describe those tumors occurring on the maxillary or mandibular mucosa. This term has largely been discarded because of etiologic and descriptive inaccuracy. It does not seem feasible that the generic term "epulis" should be still used to describe myoblastoma in this location, as suggested by Gray and Gruenfeld.

CONCLUSIONS

Seventeen cases of myoblastoma are reported and discussed. Although the recent literature contains numerous references to this tumor, it is essentially rare. The etiology of the myoblastoma is still widely discussed. In our opinion, however, it seems likely that the regeneration of injured striated muscle fibers provides an essential part of the background. There is little reason for implying the term "congenital epulis of the new-born", inasmuch as the term is inaccurate both morphologically and clinically.

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EOSINOPHILIC GRANULOMA[1]

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The findings and diagnosis of this rare case of eosinophilic granuloma represent a period of some 13 months of study and treatment. In the early stage the pathological findings were somewhat misleading and led to overlooking one of the most deciding factors in the final and correct diagnosis, the lesions in the mouth and the exfoliation of the teeth.

Eosinophilic granuloma was first described in 1940 by Otani and Ehrlich, Lichtenstein and Jaffe. It was considered by them to be an independent disease. Green and Farber in 1942 decided it was a variant of Hand-Schüller-Christian disease.

Histologically the process is an inflammatory lesion with marked granulomatous accumulation of mononuclear phagocytes, often many foreign body giant cells and variable numbers of eosinophiles. The eosinophiles may be found in overwhelming numbers. These lesions involve the flat bones, particularly the skull, ribs, and pelvis, although the long bones do not always escape. Clinically typical Hand-Schüller-Christian disease may show xanthomatoid pictures with or without eosinophilia in various lesions and eosinophilic granulomas without lipoid degeneration. Roentgenological examination reveals round, oval or irregular destructive lesions often with a "punched-out" appearance, which suggest myeloma or metastases of Hand-Schüller-Christian disease. If the jaws are affected, the teeth may become irregular and loose and may be exfoliated.

Farber revealed that in certain cases in children the proliferation of eosinophils is incomplete, and eosinophilic myelocytes may dominte the histologic reaction. The possibility of confusion with neoplastic processes of the meyloid series of cells is great, and beyond question certain of these lesions have been classified by competent observers as myelomas and myeloblastomas.

Strong suggestive evidence indicates that these syndromes represent a single disease entity, rapidly fatal in infantile forms which have been decsribed as Letterer Swies disease and reticuloendotheliolosis; chronic but still dangerous forms, because of the likelihood of cerebral and hypophyseal involvement, in early childhood (Hand-Schüller-Christian disease), and comparatively foreign in latter childhood or in the adult life, when the usual picture is that of the eosinophilic granuloma. As yet, the etiology is unknown. Recent pathological studies point strongly away from a fundamental metabolic disorder and in the direction of a specific infectious agent.

[1] Received for publication March 18, 1946.

CASE HISTORY

The patient was a 4 year old boy. At the age of 1½ years, the child's mother noticed a swelling in the left parietal region of the scalp. This continued to enlarge, and the child's gingivae became quite inflamed and tender. The child complained when eating, and the gingivae bled when the teeth were brushed.

When the child was 3 years old (July, 1944) he was seen by a physician. The enlargement was diagnosed as a sebaceuous cyst and was asperated numerous times. It failed to respond and penicillin was given over a period of 1 week. This had no effect on the diseased area. In December, 1944, he was referred to an orthopedic surgeon, who, in turn, referred the patient to a neurosurgeon. On December 27, 1944, the child was operated upon, and the diseased bone and tissues were curetted. Biopsy at this time was diagnosed as a malignant neoplasm.

Fig. 1. Frontal projection of pelvis showing destructive lesions in ilia

The area healed and, when the patient returned in January, 1945 for a check-up, radiographs taken of the long bones and pelvis disclosed destructive lesions in the ilia. (*fig. 1*) These lesions were considered to be the same as those of the operated parietal bone and x-ray therapy was used.

On February 27, 1945, radiographic examination showed a slight decrease in size of destructive areas in ilia. On April 5, 1945, radiographic examination revealed the same decrease in size of lesions in ilia and no new lesions were seen. On November 9, 1945, routine frontal projection of the pelvis showed almost complete obliteration of the destructive lesions in left ilium and a marked decrease in size of the lesion in the right ilium. During this entire course the patient's mouth had continued to grow worse. He was being treated, unsuccessfully, by a dentist for a fungus infection. The teeth had become quite loose and irregular with much denuded tissue. On November 9, 1945, lateral films which were taken of the mandible showed a relatively large defect in the alveolar margin in the region of the deciduous molars. In the right side, the first deciduous molar was

apparently absent, and practically all of the bone around the roots of the second deciduous molar had been destroyed. The tooth buds usually located beneath the deciduous molars were absent. On November 9, 1945, the patient was referred to the author. The mother exhibited a deciduous molar and a premolar bud which had exfoliated several weeks before. (*fig. 2*) Examination of the mouth showed deciduous molars with buccal and lingual roots exposed to within several millimeters of the apex. The soft tissue was inflamed and tender. All of the posterior teeth were loose and irregular. A consultation was held on November 11, 1945, and the possibility of xanthomatosis or Hand-Schüller-Christian disease was considered.

Fig. 2. Oblique film of mandible showing relatively large defect in alveolar margin in region of deciduous molars.

Laboratory examination on November 21, 1945 showed:

BLOOD		URINE	
Hb.	10.8 gm. %	Color Clear, Yellow	
Rbc.	4,450,000	S. P.	Q. N. S.
Wbc.	7,600	Reac.	Acid
Seg.	38%	Alb.	Neg.
Band.	2%	Micro:	3–5 Rbc/hpf.
Lym.	46%		occ. Wbc.
Mono.	2%		Few epith. cells
Eos.	12%	Bence-Jones	protein-neg.
Bleeding Time. 3′–30″			
Clotting Time. 4′			
Blood Calcium. 9.7 mgs. %			
Blood Cholesterol. 179 mgs.%			

In all reported cases of eosinophilic granuloma, the highest eosinophilia was only 6% as compared to this case of 12%.

Radiographic examination of the skull on November 21, 1945 by frontal and left lateral projections showed an old operative defect in the antero-inferior portion of left parietal bone. A slightly irregular rounded defect, which measured

Fig. 3. Left lateral projection of skull shows old operative defect in antero-inferior portion of left parietal bone and a slightly irregular rounded defect in the middle portion of occipital bone.

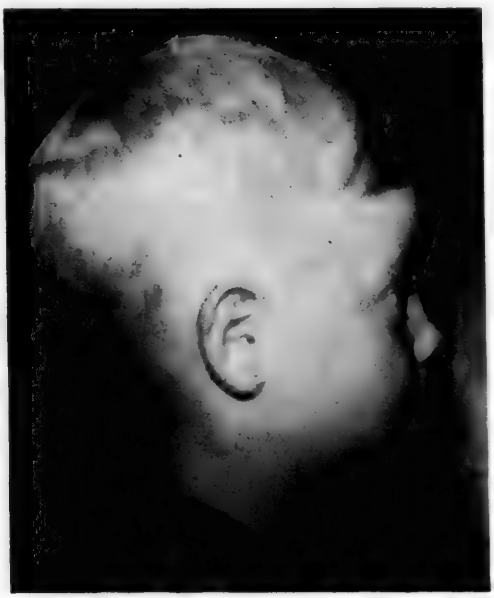

Fig. 4. Profile of patient age 4 years. Note opthalmus.

about 1 cm., was seen in the middle portion of occipital bone. Another defect was present in right parietal bone near the lamboid suture. These defects were more suggestive of xanthamotosis than of a neoplasm, although the latter could not be entirely eliminated. (*figs. 3 and 4*)

A biopsy was taken from right side of the mandible in area of first and second deciduous molars on December 5, 1945. The material consisted of 2 irregular masses of relatively firm grayish tissue. The largest mass measured 1 cm. in diameter. Diagnosis was an eosinophilic granuloma. The surface epithelium was markedly infiltrated with eosinophiles and showed superficial ulceration. The underlying tissue showed closely packed masses of large, ovoid macrophages some of which contained foamy appearing cytoplasm. Throughout the section there were massive infiltrations with eosinophilic leucocytes. Numerous young capillaries were present in the region.

The second biopsy and the finding of other cranial lesions led to the conclusion that the patient had eosinophilic granuloma of Hand-Schüller-Christian disease, not a malignant neoplasm.

Treatment. Since the pelvic lesions responded to slight x-ray therapy, x-ray radiation is being used as treatment of the jaws and other cranial lesions. Although the curettment of the mandible was done to obtain a biopsy, it was noted that the mouth as a whole showed marked improvement following curettment. X-ray examination will be continued at 3 to 4 month intervals to note progress of treatment and also to detect any new lesions so treatment may include these new lesions.

The prognosis of eosinophilic granuloma is more hopeful than that of a malignant neoplasm. X-ray will control the destructive lesions. If the occipital bone is involved, thus affecting the pituitary body, the patient may become demented and may develop diabetes insipidus. Prognosis should be made with much care because of the possibility of later visceral involvement.

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HUMAN SALIVA

XVI. Relationship of Total Calcium to Inorganic Phosphorus of Resting Saliva[1, 2]

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INTRODUCTION

Salivary calcium and inorganic phosphorus have been implicated in many ways in the normal and pathologic processes of oral physiology. Especially has it been hoped that the laws of ion concentration and the ratios of these minerals in solution would provide a key to solving the problems of maintaining oral health. The known relationships of calcium and inorganic phosphorus in other biological materials suggests the possibility of a correspondence between their levels in saliva. For this reason a study of the relationships between these minerals and their ratios and products has been made; previously reported analytical material (2, 1, 4) was used for this purpose.

RELATIONSHIP OF MILLIGRAMS PER CENT VALUES[3] OF TOTAL CALCIUM TO INORGANIC PHOSPHORUS

The individual results of calcium (1) and phosphorus (4) analyses of resting saliva of 650 individuals between 5 and 95 years of age have been arranged in a correlation grid (*fig. 1A*), which shows a tendency for low calcium values to be associated with low inorganic phosphorus values, and high calcium values with high inorganic phosphorus values. The grid is divided into quarters by heavy lines which indicate the step intervals containing the means of the total cases. Since the values are scattered widely each column of the grid was averaged and the results recorded graphically in *fig. 1B*. The individual means show a progressive rise in calcium mg.% content with increasing inorganic phosphorus values (Table I).

A definite correlation between calcium and inorganic phosphorus is indicated by the coefficient $r = +0.402 \pm 0.033$ calculated for the entire group. In order to test the influence of age on this relationship, r was calculated for the early age

[1] Presented at the 22nd General Meeting of the International Association for Dental Research, Chicago, March 18 and 19, 1944. (*J. D. Res.* **23**: 211, 1944.)

[2] Conducted under grants made by the American Foundation for Dental Science, the California State Dental Association, and the Research Board of the University of California. Received for publication April 22, 1946.

[3] The relationships between the mg./hr. values of calcium and phosphorus will be reported in number XVII of this series.

range from 5 to 29 years and was found to be unchanged ($r = +0.374 \pm 0.047$); therefore, age does not influence this relationship.

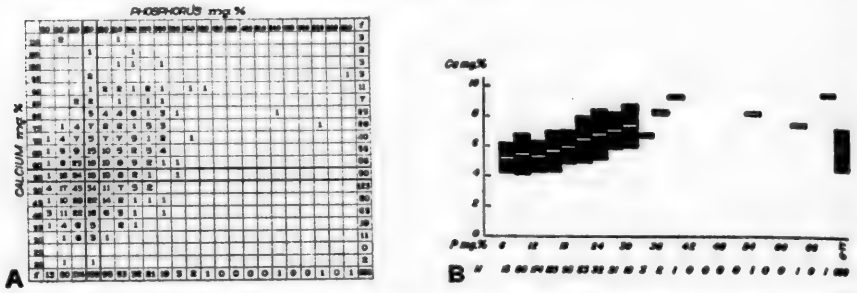

Fig. 1. (A) Distribution of total calcium mg.% values by inorganic phosphorus mg.% groups. (B) Mean ±1 standard deviation for each inorganic phosphorus mg.% interval.

TABLE I

Mean and dispersion of total calcium mg.% values of 650 healthy individuals (5 to 95 years) by inorganic phosphorus mg.% groups

PHOSPHORUS GROUPS	NUMBER OF INDIVIDUALS	CALCIUM	
		Average (Mean) $M \pm \sigma_M$	Standard Deivation $\sigma \pm \sigma_\sigma$
mg.%	*N*	*mg.%*	*mg.%*
6.0	13	5.2 ± 0.3	1.0 ± 0.2
9.0	80	5.4 ± 0.2	1.4 ± 0.1
12.0	174	5.3 ± 0.1	1.0 ± 0.05
15.0	155	5.7 ± 0.1	1.4 ± 0.1
18.0	95	5.9 ± 0.1	1.1 ± 0.1
21.0	53	6.4 ± 0.2	1.6 ± 0.2
24.0	32	6.8 ± 0.3	1.6 ± 0.2
27.0	21	7.0 ± 0.3	1.2 ± 0.2
30.0	18	7.3 ± 0.4	1.4 ± 0.2
33.0	3	6.7*	*
36.0	2	8.2	
39.0	1	9.2	
54.0†	1	8.2	
63.0	1	7.7	
69.0	1	9.7	

* σ_M, σ, and σ_σ have been omitted in the smaller groups.
† Intervals with zero frequencies have been omitted.

CALCIUM-PHOSPHORUS RATIO

The ratio[4] of calcium to phosphorus was calculated for each individual and ranged from 0.12 to 1.22 mg.% with an average of 0.36 mg.% and a standard deviation of 0.12 mg.% The frequency curves of calcium-phosphorus ratios (*figs. 2A, B,* and *C*) for the total, male, and female groups from 5 to 49 years of

[4] The ratio of mg./hr. values is identical with that of mg.%.

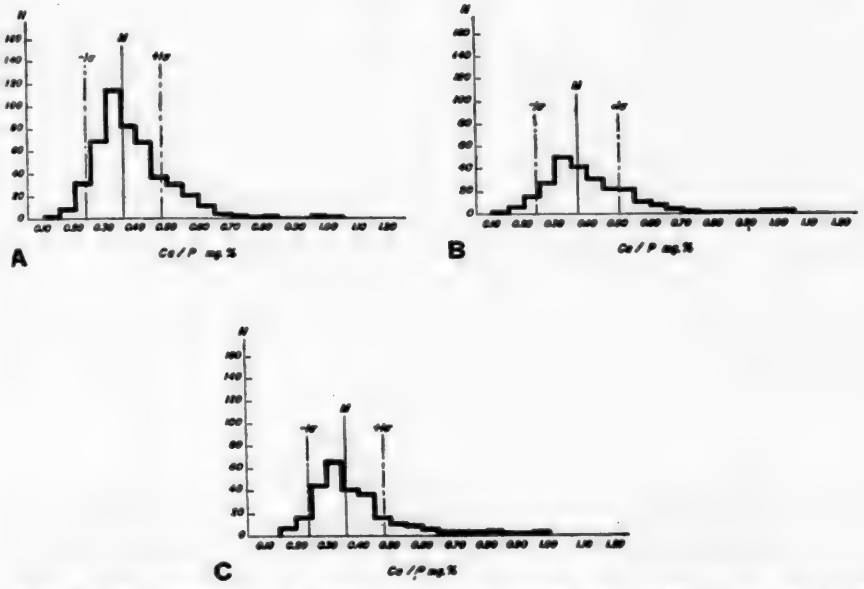

Fig. 2. Distributions of total calcium-inorganic phosphorus ratio of individuals aged 5 to 49 years. (A) 484 individuals, total group. (B) 235 males. (C) 249 females.

AGE

CALCIUM/PHOSPHORUS mg. %	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	f
1.20												1								1
1.15																				0
1.10																				0
1.05																				0
1.00				1																1
.95		1						1												2
.90																				0
.85																				0
.80		1		1																2
.75				1																1
.70			1	1			1													3
.65		1	1	1	1					1										5
.60	1	2	3	1	1	1	1			1		1								12
.55	3	4	4	3	2		2		1	1		1		1						22
.50		8	4	9	3	2	2	2	1	2	4	1		2						40
.45	2	7	3	11	2	4	2	2	4	1	3		1	1						43
.40	9	17	6	9	8	6	4	2	6	1	2	4	1			1				76
.35	12	22	13	14	4	5	2	3	7	4	4	4	1	4	2		1	1	1	104
.30	12	33	12	12	9	9	6	12	9	6	5	7	6	8	1	1				148
.25	11	18	6	13	1	4	1	7	8	7	3	12	2	2	6	2				103
.20	4	8	6	4	1	2	3	2	2	7	5	5	2	10	4					85
.15		3	1	2		1			2	1	1	1	2	4	1					19
.10							1				1				1					3
f	54	125	60	83	32	34	25	31	40	32	28	37	15	32	15	4	1	1	1	680

Fig. 3. Distribution of ratio of total calcium to inorganic phosphorus by age

age[5] are moderately symmetrical with a low skewness value ($Sk_5 = +0.25$) and resemble those of the original calcium and phosphorus mg.% distributions (1, 4). The ratios were then plotted against age as seen in *fig. 3*. There is no tendency on the part of the ratios to either decrease or increase with advancing age.

Since the values in this grid are so widely spread, means and standard deviations were calculated for each individual column of *fig. 3*. The lines of means of calcium-phosphorus ratios, (*figs. 4A*, *B*, and *C*) for total, male and female groups, show a progressive increase with age from 5 up to approximately 29 years and subsequently a decline with the lowest values in the oldest age groups.

The correlation figure ($r = -0.241 \pm 0.037$) which is calculated on the basis of the entire group from 5 to 95 years, indicates a slight tendency for the calcium-phosphorus ratio to *decrease* as age advances. That this tendency expresses only part of the picture is seen by the fact that the correlation coefficient of the

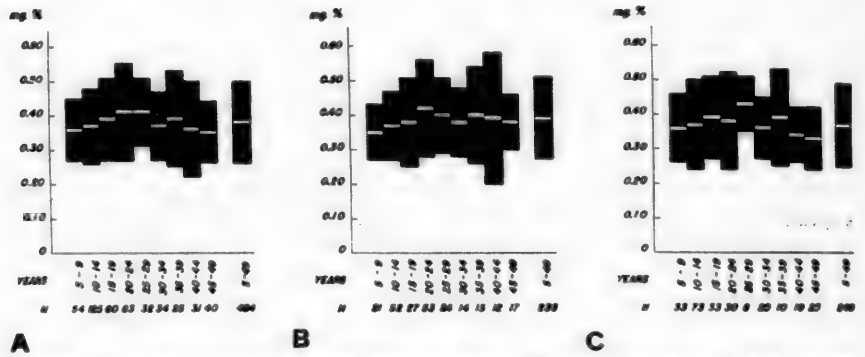

Fig. 4. Total calcium-inorganic phosphorus ratio of individuals aged 5 to 49 years. Mean ±1 standard deviation for each 5 year interval of (A) 484 individuals, total group, (B) 235 males, and (C) 249 females.

younger individuals from 5 to 29 years of age is found to be $+0.268 \pm 0.036$ which indicates that in these early age groups the ratio *increases* with age.

CALCIUM-PHOSPHORUS PRODUCTS

The *mg.%*products of total calcium and inorganic phosphorus show a wide scattering ranging from 27.2 to 681.6 for the entire group. The frequency curves (*figs. 5A*, *B*, and *C*) show a tailing off toward higher values and consequently a large skewness value ($Sk_5 = +0.62$). It appears, therefore, that comparison of averages and standard deviations is not feasible, as it would be in the case of a symmetrical distribution. For this reason the values have been omitted. The products were arranged by five year age groups as seen in the grid of *fig. 6* The correlation coefficient ($r = +0.325 \pm 0.035$) calculated from the of the 5 to 95 year group is slight, while in the 5 to 29 year group it is greater ($r = +0.445 \pm 0.031$) perhaps because of less scattering of value (*fig. 6*).

[5] See reference 2 for the selection of this age group.

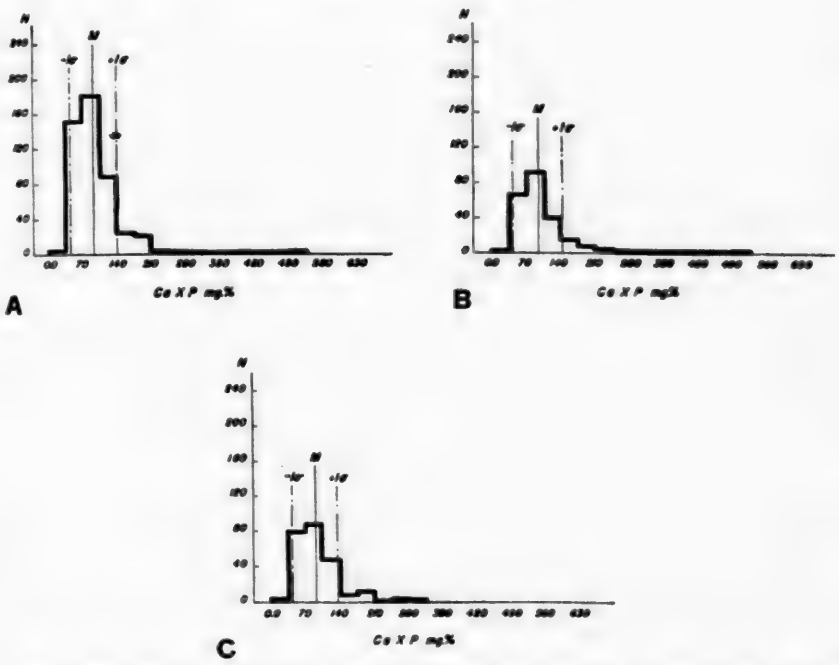

Fig. 5. Distributions of total calcium-inorganic phosphorus product mg.% of individuals aged 5 to 49 years. (A) 484 individuals, total group. (B) 235 males. (C) 249 females.

AGE

CALCIUM X PHOSPHORUS mg %	5	10	15	20	25*	30	35	40	45	50	55	60	65	70	75	80	85	90	95	f
665											1									1
630																				0
595																				0
560																				0
525																				0
490							1													1
455																				0
420										1										1
385																				0
350												1								1
315												1								1
280								1		1	1	1								4
245				1			1	1	2			4			1					10
210				2			1		2	3	1	2		3						14
175		1	3	5	3	4	3	2		2	3	2	1	6	1					36
140	3	3	4	3		1	2		8	4	6	4	2	4	2		1			47
105	2	14	14	14	10	9	4	11	13	7	5	8	3	1	6	2				123
70	19	58	16	33	14	12	6	12	11	7	7	7	6	10	2	1			1	222
35	29	47	23	24	4	8	7	4	4	7	4	6	3	8	3	1		1		183
0	1	2		1	1							1								6
f	54	125	60	83	32	34	25	31	40	32	28	37	15	32	15	4	1	1	1	650

Fig. 6. Distribution of total calcium-inorganic phosphorus product mg.% by age

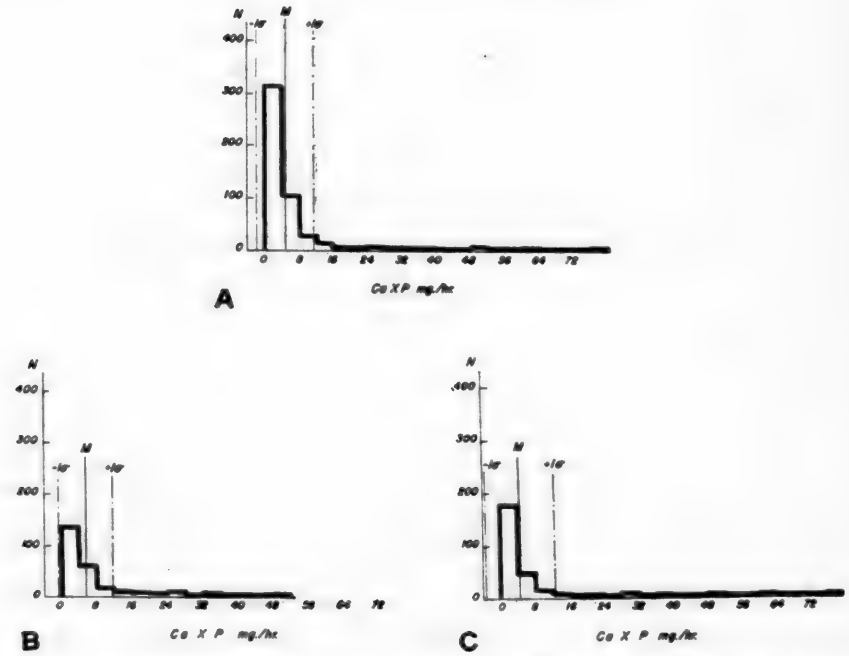

Fig. 7. Distributions of total calcium-inorganic phosphorus product mg./hr. of individuals aged 5 to 49 years. (A) 484 individuals, total group. (B) 235 males. (C) 249 females.

AGE

CALCIUM X PHOSPHORUS mg./hr.	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	f
76				1																1
72																				0
68																				0
64																				0
60			1																	1
56																				0
52																				0
48		1			1															2
44																				0
40											1									1
36		1																		1
32				1	1															2
28			1			1						2								4
24		1		1	1			1				1								5
20					1	1			1	1										4
16		1		1			1			2	1			2						8
12	1	1	3	4	1		1	1	2	2	2	1	1							20
8		6	6	4	1	5	3	2	3	2	1	2		3	1					39
4	7	27	7	21	9	10	5	7	13	8	8	4	5	7	1					139
0	46	87	42	50	17	17	15	20	21	17	15	27	9	20	13	4	1	1	1	423
f	54	125	60	83	32	34	25	34	40	32	28	37	15	32	15	4	1	1	1	650

Fig. 8. Distribution of total calcium-inorganic phosphorus product mg./hr. by age.

The *mg./hr.* products of calcium and phosphorus also are widely scattered with a range of 0.07 to 77.25. An extremely skewed distribution ($Sk_s = +1.30$) is seen in the frequency curves (*fig. 7*). Again the averages and standard deviations of five year groups were not calculated because of the extreme asymmetry. The products were arranged by five year intervals (*fig. 8*) but no correlation could be found with age for the total group ($r = -0.027 \pm 0.039$) or for the 5 to 29 year group, ($r = +0.284 \pm 0.036$). Since the first step interval of the grid contains 423 of the total of 650 cases this group was divided further into 0.5 intervals. Table II confirms the extreme asymmetry (5) of the distribution.

TABLE II

Frequency distribution of total calcium-inorganic phosphorus product (mg./hr.) of the first step interval (0 to 3.5 mg./hr.) of figure 8

Ca × P MG./HR. GROUPS	NUMBER OF INDIVIDUALS
	N
0.0	76
0.5	70
1.0	79
1.5	56
2.0	46
2.5	48
3.0	30
3.5	18
N.....	423

SUMMARY AND CONCLUSIONS

The relationships between total calcium and inorganic phosphorus were examined in the analyses of resting saliva from 650 healthy individuals 5 to 95 years of age, and may be summarized as follows:

1. Calcium and phosphorus mg.% levels were directly related in all age groups ($r = +0.402 \pm 0.033$); that is, low values were associated as also were high values. This means that like values (i.e., above or below the mean) of calcium and phosphorus were found together in 63% of cases (3).

2. The average ratio of total calcium to inorganic phosphorus was 0.36 with a standard deviation of 0.12. The lowest and highest values observed were 0.12 and 1.22. The distributions were moderately symmetrical with a skewness value (Sk_s) of +0.25 and a coefficient of variation (V_1) of 33.

3. The calcium-phosphorus ratio (which is numerically the same for both mg.% and mg./hr. values) was found to vary among individuals of different ages, with the highest ratios between 20 and 29 years and the lowest in younger and older groups. Averages of 5 year groups were found to rise and to fall progressively to and from the peak ratio. The changes follow the direction anticipated from the evaluation of calcium and phosphorus mg.% findings in relation to age. The fact that the ratio changes in relation to age indicates a shift in the propor-

tions of the various calcium and inorganic phosphorus containing compounds of saliva. As a child grows the salivary calcium and phosphorus content both increase; however, the calcium content increases more rapidly. Between 20 and 29 years, shortly after maturity is reached, not only is the highest percentage concentration of calcium observed but the proportion of calcium to phosphorus is at its lifetime peak. At later ages the calcium content does not increase perceptibly while the phosphorus content continues to rise, this change leads to a decrease in the calcium-phosphorus ratio in adult and later life.

4. The mg.% products of calcium and phosphorus were lowest in the youngest age groups as would be expected on the basis of the observed changes in the ratios. The products varied widely among different individuals as expressed in the range (27.2 to 681.6), in the extreme skewness of the distribution (Sk_5 = +0.62), and in the high coefficient of variation (V_1 = 58).

5. The mg./hr. products of total calcium and inorganic phosphorus varied greatly among different individuals with a wide range from 0.07 to 77.25, extreme asymmetry of distributions, high skewness value (Sk_5 = +1.30) and large coefficient of variation (V_1 = 138). No difference was found between five year age groups.

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HUMAN SALIVA

XVII. Relationship of Total Calcium and Inorganic Phosphorus to Rate of Flow of Resting Saliva[1, 2]

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INTRODUCTION

A dependence of salivary composition upon rate of flow has been recognized (1, 2, 9. 10, 11, 14, 16); in many studies, however, its possible effect on the results has been ignored (12, 13, 17, 20). Salivary composition is altered markedly by paraffin activation, because of the increased rate of flow (5, 7, 8, 15, 19). Analyses of resting saliva from different individuals and from the same individual on different days show significant differences in group averages of calcium and phosphorus content in extreme rate of flow groups (4). Great variation in the relationship between rate of flow and composition seems to exist which indicates that the relationship is complex. For the study of this relationship a further evaluation of the resting saliva values of 650 individuals previously reported in this series (6, 3, 18) was made.

RELATIONSHIP OF TOTAL CALCIUM AND INORGANIC PHOSPHORUS MILLIGRAMS PER CENT AND MILLIGRAMS PER HOUR VALUES TO RATE OF FLOW

The distributions of total calcium and inorganic phosphorus *mg.%* values have been arranged by rate of flow intervals of 5 cc./hr. in *fig. 1A* and *B*. The grids are divided into quarters by the heavy lines which indicate the step intervals containing the means of the 650 cases. Fewer observations are noted in the lower left and upper right (opposite) quadrants indicating a slight tendency for mg.% values to be related inversely to rate of flow. Since the individual values are scattered so widely, the columns have been averaged and standard deviations and sampling errors calculated (Table I and *figs. 2A* and *B*). In the graphs and tables progressively lower mg.% values of calcium and phosphorus are seen as rate of flow increases from 0 to approximately 19 cc./hr. while in the higher rates of flow the composition is quite uniform. The correlation coefficients fail to show significant relationships since for calcium mg.% r is -0.156 ± 0.038, and for phosphorus -0.281 ± 0.034. When only the slower rate of flow groups

[1] Presented at the 22nd General Meeting of the International Association for Dental Research, Chicago, March 18 and 19, 1944. (*J. D. Res.* **23**: 212, 1944.)

[2] Conducted under grants made by the American Foundation for Dental Science, the California State Dental Association, and the Research Board of the University of California. Received for publication April 22, 1946.

from 0 to 19 cc./hr. are considered the relationship is not found to be significant; for calcium R = -0.187 ± 0.048 and for phosphorus -0.202 ± 0.047.

The averages of rate of flow groups (Table I) which were calculated from *figs. 1C* and *D* show a strong relationship between both calcium mg./hr. and phosphorus mg./hr. and rate of flow with a correlation coefficient of r = $+0.913$

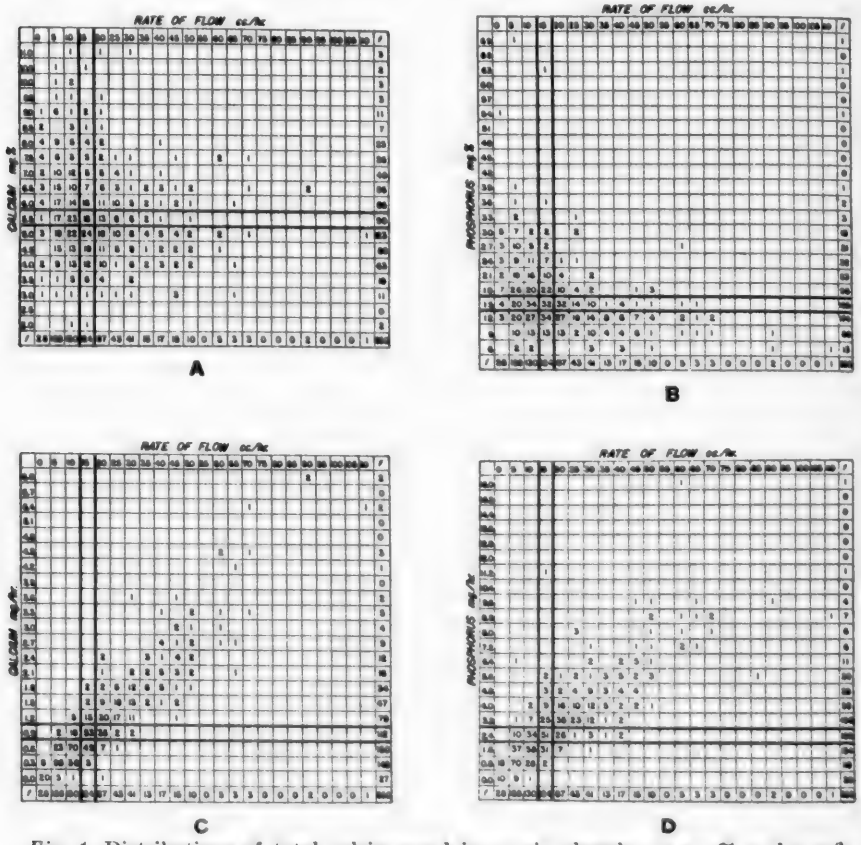

Fig. 1. Distributions of total calcium and inorganic phosphorus mg.% and mg./hr. values by rate of flow groups. (A) Ca mg.%. (B) P mg.%. (C) Ca mg./hr. (D) P mg./hr.

± 0.006 and $+0.818 \pm 0.013$, respectively. The relationship is demonstrated graphically in *figs. 2C* and *D*.

RELATIONSHIP OF TOTAL CALCIUM MILLIGRAMS PER HOUR TO INORGANIC PHOSPHORUS MILLIGRAMS PER HOUR

A strong relationship is observed between calcium and phosphorus mg./hr., r = $+0.783 \pm 0.016$ (*fig. 3A* and *B* and Table II). In order to explain the basis on which this high correlation occurs, it is necessary to re-examine the relation-

TABLE I

Mean and dispersion of total calcium and inorganic phosphorus mg.% and mg./hr. values of 650 individuals (5 to 95 years) by rate of flow groups

RATE OF FLOW GROUPS	NUMBER OF INDIVIDUALS	CALCIUM				PHOSPHORUS			
		mg.%		mg./hr.		mg.%		mg./hr.	
cc./hr.	N	$M \pm \sigma_M$	$\sigma \pm \sigma_\sigma$	$M \pm \sigma_M$	$\sigma \pm \sigma_\sigma$	$M \pm \sigma_M$	$\sigma \pm \sigma_\sigma$	$M \pm \sigma_M$	$\sigma \pm \sigma_\sigma$
0	28	6.6 ±0.3	1.6 ±0.2	0.2 ±0.02	0.1 ±0.01	23.2 ±1.5	8.6 ±1.2	0.9 ±0.1	0.4±0.05
5	128	6.2 ±0.1	1.5 ±0.1	0.4 ±0.02	0.2 ±0.01	19.9 ±0.7	7.9 ±0.5	1.6 ±0.1	0.8±0.05
10	130	5.9 ±0.1	1.5 ±0.1	0.7 ±0.02	0.2 ±0.01	17.3 ±0.5	5.2 ±0.3	2.2 ±0.1	0.7±0.04
15	124	5.6 ±0.1	1.3 ±0.1	0.9 ±0.02	0.2 ±0.01	17.1 ±0.6	6.5 ±0.4	3.0 ±0.1	1.2±0.1
20	87	5.6 ±0.2	1.4 ±0.1	1.2 ±0.04	0.4 ±0.03	14.9 ±0.4	3.3 ±0.3	3.4 ±0.1	0.8±0.1
25	43	5.8 ±0.1	0.9 ±0.1	1.5 ±0.03	0.2 ±0.02	16.0 ±0.8	5.1 ±0.5	4.4 ±0.2	1.3±0.1
30	41	5.3 ±0.2	1.3 ±0.1	1.7 ±0.05	0.3 ±0.03	13.3 ±0.6	3.5 ±0.4	4.4 ±0.2	1.1±0.1
35	13	5.5 ±0.2	0.8 ±0.2	2.0 ±0.1	0.3 ±0.1	12.3 ±0.5	1.7 ±0.3	4.8 ±0.3	0.9±0.2
40	17	5.6 ±0.3	1.1 ±0.2	2.3 ±0.1	0.5 ±0.1	11.9 ±0.8	3.1 ±0.5	5.1 ±0.3	1.2±0.2
45	15	5.0 ±0.3	1.3 ±0.2	2.4 ±0.2	0.6 ±0.1	12.4 ±0.6	2.5 ±0.5	6.2 ±0.4	1.4±0.3
50	10	5.4 ±0.3	0.9 ±0.2	2.7 ±0.2	0.5 ±0.1	14.2 ±1.2	3.8 ±0.8	7.4 ±0.5	1.7±0.4
60*	5	6.1 ±0.6	1.3 ±0.4	3.7 ±0.4	0.8 ±0.3	16.0 ±2.8	6.3 ±2.0	9.8 ±1.5	3.3±1.0
65	3	4.5 ±0.7	1.2 ±0.5	3.1 ±0.5	0.8 ±0.4	13.0 ±1.4	2.5 ±1.0	8.9 ±0.6	1.0±0.4
70	3	6.5 ±0.6	1.0 ±0.4	4.5 ±0.5	0.9 ±0.4	12.0 ±0.8	1.4 ±0.6	8.9 ±0.2	0.4±0.2
90	2	6.7 ±0.0	0.0 ±0.0	6.1 ±0.0	0.0 ±0.0	8.5 ±1.1	1.5 ±0.8	8.0 ±1.4	2.0±1.0
110	1	5.2 ±0.0	0.0 ±0.0	5.5 ±0.0	0.0 ±0.0	7.0 ±0.0	0.0 ±0.0	9.2 ±0.0	0.0±0.0

* Intervals with zero frequencies have been omitted.

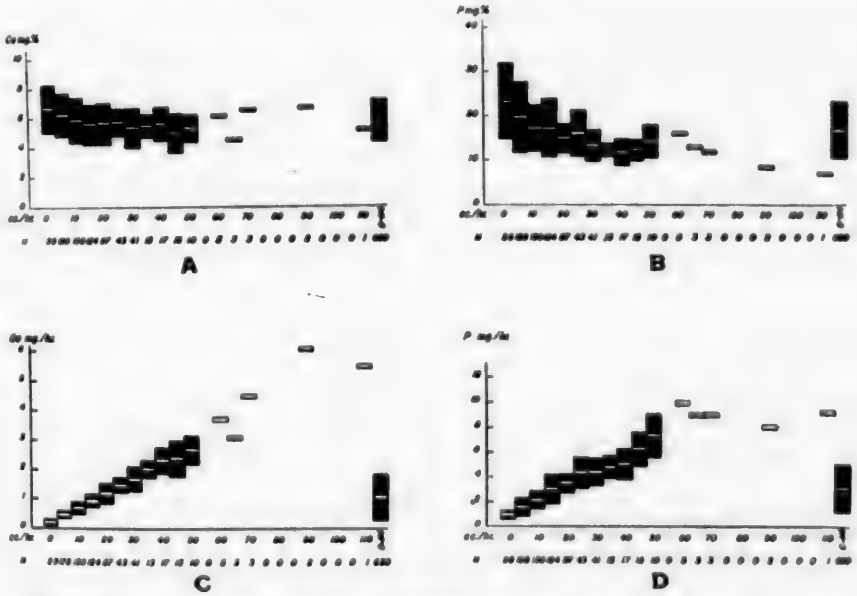

Fig. 2. Total calcium and inorganic phosphorus mg.% and mg./hr. values showing mean ±1 standard deviation for each rate of flow interval. (A) Ca mg.%. (B) P mg.%. (C) Ca mg./hr. (D) P mg./hr.

ships between calcium mg.% and rate of flow, and phosphorus mg.% and rate of flow (*figs. 1A* and *B*). Although their group averages differ in the lower rate of flow levels, this difference due to rate of flow is not significant because of the

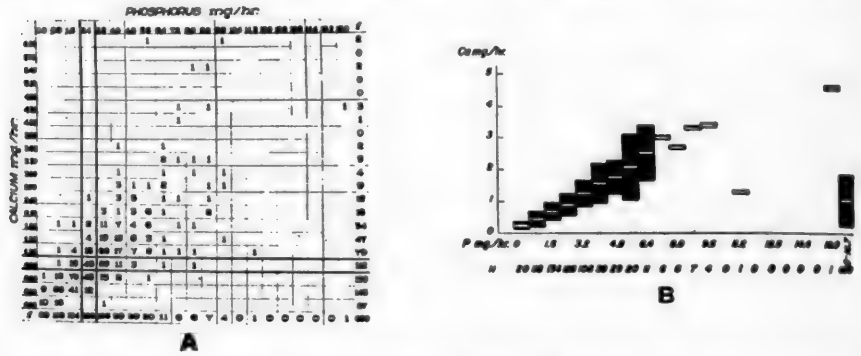

Fig. 3. (A) Distribution of total calcium mg./hr. values by inorganic phosphorus mg./hr. groups. (B) Mean ±1 standard deviation for each inorganic phosphorus mg./hr. interval.

TABLE II

Mean and dispersion of total calcium mg./hr. values of 650 individuals (5 to 95 years) by inorganic phosphorus mg./hr. groups

PHOSPHORUS GROUPS	NUMBER OF INDIVIDUALS	CALCIUM	
		Average (Mean) M ± σ_M	Standard Deviation $\sigma \pm \sigma_\sigma$
mg./hr.	*N*	*mg./hr.*	*mg./hr.*
0.0	20	0.3 ± 0.02	0.1 ± 0.02
0.8	118	0.4 ± 0.02	0.2 ± 0.01
1.6	134	0.7 ± 0.02	0.2 ± 0.01
2.4	128	0.9 ± 0.03	0.3 ± 0.02
3.2	106	1.3 ± 0.04	0.4 ± 0.03
4.0	59	1.6 ± 0.1	0.6 ± 0.1
4.8	29	1.8 ± 0.1	0.5 ± 0.1
5.6	20	2.1 ± 0.2	1.0 ± 0.2
6.4	11	2.5 ± 0.3	0.9 ± 0.2
7.2	6	3.0 ± 0.5	1.2 ± 0.3
8.0	6	2.7 ± 0.6	1.5 ± 0.4
8.8	7	3.3 ± 0.4	1.2 ± 0.3
9.6	4	3.4 ± 0.8	1.7 ± 0.6
11.2*	1	1.3 ± 0.0	0.0 ± 0.0
16.0	1	4.6 ± 0.0	0.0 ± 0.0

* Intervals with zero frequencies have been omitted.

rather great spread of the values. In other words, among *different individuals*, the resting saliva mg.% values of calcium and phosphorus vary independently of the rate of flow. On the other hand, the mg./hr. values are greatly affected

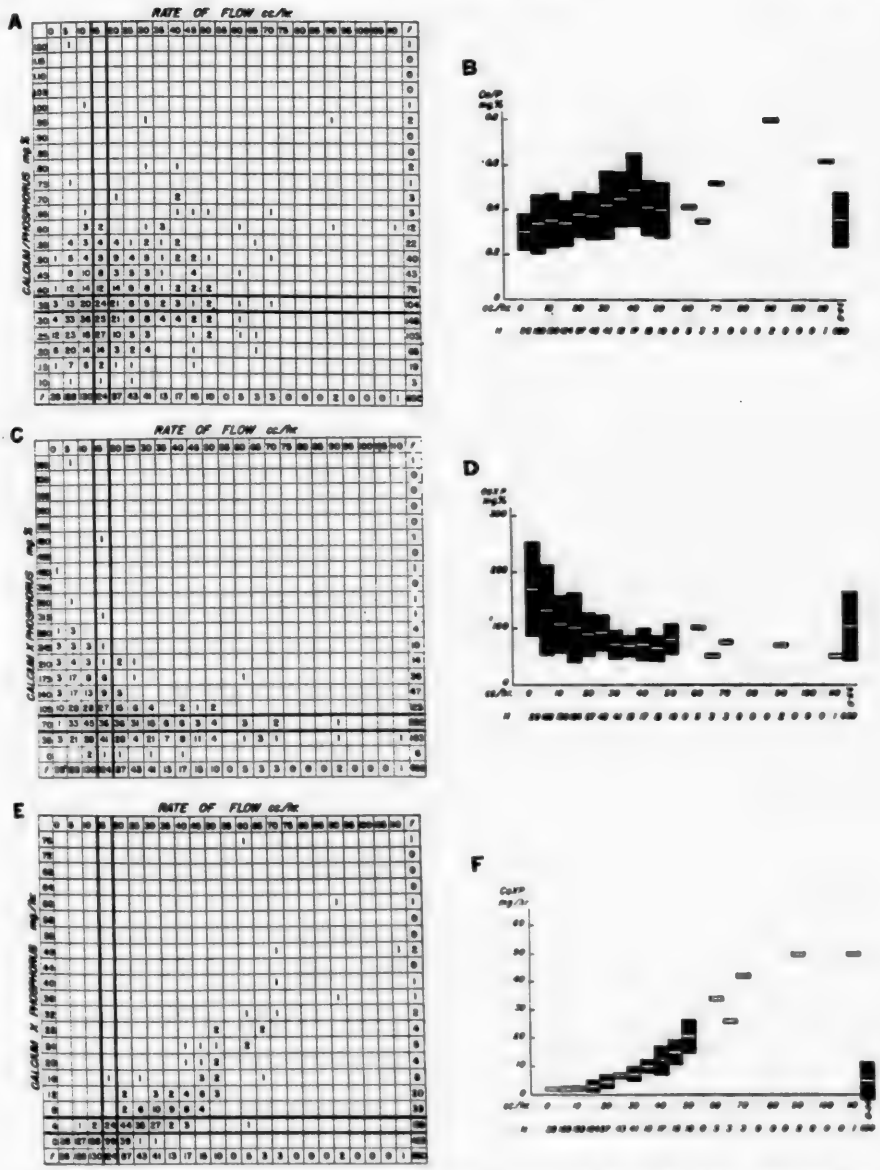

Fig. 4. Distributions of total calcium-inorganic phosphorus ratio and products by rate of flow groups, (A) Ca/P mg.%, (C) CaXP mg.%, (E) CaXP mg./hr. Mean ±1 standard deviation for each rate of flow interval, (B) Ca/P mg.%, (D) CaXP mg.%, (F) CaXP mg./hr.

by the rate of flow, cc./hr. (*fig. 1C* and *D*). This means that the mg.% concentrations of calcium and phosphorus of different individuals are relatively constant

at different resting saliva rates of flow; however, since mg.% concentrations are relatively constant the total quantity (e.g., calcium) produced in a given time unit (mg./hr.) varies directly with the amount of saliva secreted (cc./hr.).

TABLE III

Mean and dispersion of total calcium-inorganic phosphorus ratio and products of 650 individuals (5 to 95 years) by rate of flow groups

RATE OF FLOW GROUPS	NUMBER OF INDIVIDUALS	Ca/P		Ca × P					
				mg.%				mg./hr.	
cc./hr.	N	$M \pm \sigma_M$	$\sigma \pm \sigma_\sigma$	$M \pm \sigma_M$		$\sigma \pm \sigma_\sigma$		$M \pm \sigma_M$	$\sigma \pm \sigma_\sigma$
0	28	0.3 ± 0.02	0.1 ± 0.01	168.2 ±	15.1	83.6 ±	11.3	2.0 ± 0.0	0.0 ± 0.0
5	128	0.3 ± 0.01	0.1 ± 0.01	132.4 ±	7.0	79.1 ±	4.9	2.0 ± 0.04	0.4 ± 0.03
10	130	0.4 ± 0.01	0.1 ± 0.01	106.6 ±	4.4	50.4 ±	3.1	2.1 ± 0.04	0.5 ± 0.03
15	124	0.3 ± 0.01	0.1 ± 0.01	100.6 ±	5.4	60.9 ±	3.9	2.9 ± 0.2	2.1 ± 0.1
20	87	0.4 ± 0.01	0.1 ± 0.01	88.2 ±	4.0	37.4 ±	2.8	4.5 ± 0.3	2.6 ± 0.2
25	43	0.4 ± 0.02	0.1 ± 0.01	94.3 ±	4.7	30.8 ±	5.5	6.7 ± 0.1	0.6 ± 0.07
30	41	0.4 ± 0.03	0.2 ± 0.02	70.8 ±	3.8	24.5 ±	2.7	7.5 ± 0.4	2.6 ± 0.3
35	13	0.4 ± 0.03	0.1 ± 0.02	68.2 ±	4.9	17.5 ±	3.5	10.0 ± 0.7	2.4 ± 0.5
40	17	0.5 ± 0.05	0.2 ± 0.03	70.5 ±	17.0	27.3 ±	4.7	11.9 ± 1.2	5.2 ± 0.9
45	15	0.4 ± 0.03	0.1 ± 0.02	63.7 ±	5.2	21.0 ±	3.8	15.1 ± 1.2	4.5 ± 0.8
50	10	0.4 ± 0.03	0.1 ± 0.02	80.0 ±	8.2	26.2 ±	5.9	20.8 ± 1.9	6.0 ± 1.3
60*	5	0.4 ± 0.1	0.2 ± 0.06	101.0 ±	21.4	47.6 ±	15.0	34.0 ±	10.7 23.9 ± 7.6
65	3	0.4 ± 0.1	0.2 ± 0.08	52.0 ±	0.0	0.0 ±	0.0	26.0 ± 3.2	5.6 ± 2.3
70	3	0.5 ± 0.1	0.1 ± 0.04	75.3 ±	9.4	16.4 ±	6.7	42.0 ± 6.2	10.8 ± 4.4
90	2	0.8 ± 0.1	0.1 ± 0.05	69.5 ±	12.3	17.5 ±	8.7	50.0 ± 8.4	12.0 ± 6.0
110	1	0.6 ± 0.0	0.0 ± 0.0	52.0 ±	0.0	0.0 ±	0.0	50.0 ± 0.0	0.0 ± 0.0

* Intervals with zero frequencies have been omitted.

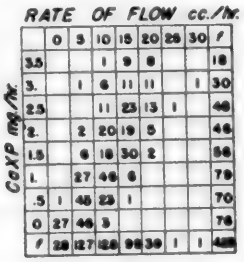

Fig. 5. Distribution of total calcium-inorganic phosphorus product from 0 to 3.5 mg./hr. by rate of flow groups.

Thus the values of mg./hr. are dependent on the values of cc./hr., both being expressions of quantity. This conclusion is substantiated by the calculation of the partial correlation coefficient of the relation between calcium and phosphorus mg./hr. holding rate of flow constant, $r_{12.3} = +0.154 \pm 0.038$, which is negligible.

Therefore, *any* combination of expressions of quantity per hour (mg./hr. or cc./hr.) will give a high correlation coefficient.

RELATIONSHIP OF CALCIUM-PHOSPHORUS RATIO AND PRODUCTS TO RATE OF FLOW

The calcium-phosphorus *ratio*[3] (*fig. 4A* and *B*, and Table III) shows a very slight relationship to rate of flow, r is $+0.237 \pm 0.037$. Generally speaking, then, as the rate of flow decreases the phosphorus mg.% level increases at a faster rate than calcium. This correlation is greater in the younger persons from 5 to 29 years where r is $+0.396 \pm 0.045$.

The *mg.% product* of calcium and phosphorus shows a slight inverse relation to rate of flow as can be seen in *fig. 4C* and *D* and Table III; the correlation is very low ($r = -0.223 \pm 0.037$) and reflects the relationships of mg.% calcium and phosphorus to rate of flow.

The *mg./hr. product* of calcium and phosphorus is closely related to rate of flow, $r = +0.770 \pm 0.016$, as would be expected for measures of quantity (*fig. 4E* and *F*, and Table III). Since 423 of the 650 individuals of the distribution (*fig. 4E*) are found in the first step interval, a grid was constructed to test the relationship to rate of flow if smaller intervals were taken in this group. In *fig. 5* the products have been grouped by 0.5 mg./hr. intervals from 0.0 to 3.9 mg./hr. and demonstrates the same relation to rate of flow as that seen in the total group. The high correlation could have been anticipated on the basis of the discussed relationships of any mg./hr. and cc./hr. values.

SUMMARY AND CONCLUSIONS

The relationships of total calcium and inorganic phosphorus content to rate of flow of resting saliva were examined in 650 healthy individuals and are summarized as follows:

1. The calcium content (mg.%) tended to be related inversely to rate of flow, however, with a low correlation coefficient, $r = -0.156 \pm 0.038$. The lowest rates of flow were accompanied by the highest mg.% calcium values, while above 19 cc./hr. the calcium content plateaued.

2. Similarly, the phosphorus content (mg.%) showed a slight inverse relationship to rate of flow ($r = -0.281 \pm 0.034$).

3. The correlation coefficients of all relationships between mg./hr. values (calcium or phosphorus or their ratio or products) and cc./hr. were extremely high; for example: total calcium mg./hr. and rate of flow $r = +0.913 \pm 0.006$; inorganic phosphorus mg./hr. and rate of flow, $r = +0.810 \pm 0.013$; calcium-phosphorus product mg./hr. and rate of flow, $r = +0.770 \pm 0.016$; calcium mg./hr. and phosphorus mg./hr., $r = +0.783 \pm 0.016$. These high correlations result only because of the relatively slight relation between mg.% concentration and rate of flow and the fact that the amount per time unit (mg./hr.) is greatly influenced by the amount of saliva produced (cc./hr.). That is, the values of

[3] mg.% and mg./hr. values have the same ratio.

mg./hr. are dependent on the cc./hr., as might be expected since both are expressions of quantity. This conclusion, that the high correlation between calcium and phosphorus mg./hr. values is not due to a high correlation between the minerals but is due to the manner of calculating the mg./hr. value, was confirmed in the finding that the partial correlation coefficient of calcium mg./hr. and phosphorus mg./hr. holding rate of flow constant was exceedingly low, $r_{12.3} = +0.154 \pm 0.038$.

4. The calcium-phosphorus ratio (which is numerically the same for mg.% and mg./hr. values) was related slightly to rate of flow; $r = +0.237 \pm 0.037$ indicating that the lower the rate of flow, the higher will be the calcium-phosphorus ratio.

5. The calcium-phosphorus product mg.% reflected the relationships of these constituents (considered separately) to rate of flow; r was very low, -0.223 ± 0.037.

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HUMAN SALIVA

XVIII. Is There a Seasonal Influence on Rate of Flow and Calcium and Phosphorus Content of Resting Saliva?[1, 2]

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The possibility of seasonal influences upon salivary calcium levels has been mentioned in a preliminary report of Brawley and Sedwick (4). Seasonal effects on dental caries activity also have been suspected by several authors (11, 8, 9, 10, 12, 13). Since the heat regulating mechanism of the body and other general physiologic conditions are greatly affected by climatic variations, such as those of humidity, temperature, air pressre, etc., and food intake; it is quite conceivable that not only dental caries activity but also salivary composition might be affected.

The results of a large series of salivary analyses accumulated over a 3 year period, which showed definite relations between the age of subjects, rate of flow, total calcium and inorganic phosphorus content, were evaluated for possible seasonal variations. Because of the large number of individuals examined it was felt that seasonal effects could be noted although different individuals were examined in each season.

Age: The results of 650 salivary analyses previously reported (2, 1, 14) were sorted according to the date of analysis and the values obtained over the 3 year period combined by months. Since it was found in previous studies (1, 14) that age influences calcium and phosphorus composition this factor had to be taken into consideration in comparing the values from month to month. The monthly average ages (Table I) grouped together and separated as to sex are presented graphically in *figs. 1A*, *B* and *C*. The highest average age (56 years) occurred in the month of August and the lowest (18 years) in October and otherwise they vary considerably.

Rate of Flow: The averages of rate of flow (Table I and *figs. 1D*, *E* and *F*) show a singular lack of variation by months and the standard deviations are often quite large. In Table II are given the month of analysis, age, and sex of the eleven individuals (2) who because of their low rates of flow (below 0.5 cc./hr.) are not included in the figures or tables and their samples were not sufficient for analysis.

Calcium and Phosphorus mg.% and mg./hr.: The *calcium mg.%* levels shown in *figs. 2A*, *B* and *C* are remarkably constant throughout the year. An exception

[1] Presented at the 22nd General Meeting of the International Association for Dental Research, Chicago, March 18 and 19, 1944. (*J. D. Res.* **23**: 220, 1944.)

[2] Conducted under grants made by the American Foundation for Dental Science, the California State Dental Association, and the Research Board of the University of California. Received for publication April 22, 1946.

TABLE I

Mean and dispersion of salivary total calcium, inorganic phosphorus, rate of flow, and age of 650 healthy individuals (5 to 95 years) arranged according to the month in which sample was analyzed

MONTH	NUMBER OF INDIVIDUALS	AGE		RATE OF FLOW		CALCIUM				PHOSPHORUS			
		Years		cc./hr.		mg.%		mg./hr.		mg.%		mg./hr.	
	N	$M \pm \sigma_M$	$\sigma \pm \sigma_\sigma$	$M \pm \sigma_M$	$\sigma \pm \sigma_\sigma$	$M \pm \sigma_M$	$\sigma \pm \sigma_\sigma$	$M \pm \sigma_M$	$\sigma \pm \sigma_\sigma$	$M \pm \sigma_M$	$\sigma \pm \sigma_\sigma$	$M \pm \sigma_M$	$\sigma \pm \sigma_\sigma$
Jan.	26	20 ± 3	14 ± 2	25 ± 5	24 ± 3	5.8 ± 0.3	1.5 ± 0.2	1.4 ± 0.3	1.3 ± 0.2	17.5 ± 2.3	11.8 ± 1.6	3.9 ± 0.6	3.3 ± 0.4
Feb.	83	23 ± 1	11 ± 1	20 ± 1	10 ± 1	5.8 ± 0.2	1.4 ± 0.1	1.1 ± 0.1	0.6 ± 0.1	15.5 ± 0.8	7.0 ± 0.5	3.1 ± 0.2	1.8 ± 0.1
March	27	23 ± 3	14 ± 2	19 ± 2	12 ± 2	6.0 ± 0.4	1.9 ± 0.3	1.1 ± 0.2	0.8 ± 0.1	17.0 ± 0.9	4.9 ± 0.7	3.2 ± 0.3	1.6 ± 0.2
April	50	31 ± 2	18 ± 2	21 ± 2	15 ± 2	5.7 ± 0.2	1.4 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	15.4 ± 0.6	4.4 ± 0.4	3.2 ± 0.3	1.9 ± 0.2
May	74	45 ± 2	18 ± 1	20 ± 2	18 ± 1	5.8 ± 0.2	1.6 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	18.8 ± 0.8	7.0 ± 0.6	3.4 ± 0.2	2.1 ± 0.2
June	107	27 ± 2	21 ± 1	18 ± 1	12 ± 1	5.7 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	0.7 ± 0.5	16.4 ± 0.5	4.9 ± 0.3	2.9 ± 0.2	1.8 ± 0.1
July	117	39 ± 2	24 ± 2	19 ± 1	14 ± 1	5.7 ± 0.1	1.4 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	16.5 ± 0.5	5.8 ± 0.4	3.0 ± 0.2	1.8 ± 0.1
Aug.	29	56 ± 3	16 ± 2	19 ± 3	14 ± 2	7.0 ± 0.4	1.9 ± 0.3	1.2 ± 0.1	0.7 ± 0.1	20.2 ± 1.2	6.4 ± 0.8	3.5 ± 0.4	1.9 ± 0.3
Sept.	27	30 ± 3	18 ± 2	15 ± 1	7 ± 1	5.9 ± 0.2	1.0 ± 0.1	0.9 ± 0.1	0.4 ± 0.1	16.8 ± 1.0	5.2 ± 0.7	2.4 ± 0.2	1.2 ± 0.2
Oct.	33	18 ± 2	10 ± 1	16 ± 2	9 ± 1	5.8 ± 0.2	1.2 ± 0.2	0.9 ± 0.1	0.5 ± 0.1	15.3 ± 0.9	5.0 ± 0.6	2.4 ± 0.2	1.4 ± 0.2
Nov.	27	20 ± 2	9 ± 1	18 ± 2	11 ± 2	6.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	15.3 ± 0.9	4.6 ± 0.6	2.5 ± 0.2	1.2 ± 0.2
Dec.	50	50 ± 4	25 ± 3	19 ± 2	12 ± 1	5.5 ± 0.2	1.1 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	18.6 ± 0.8	5.9 ± 0.6	3.3 ± 0.2	1.8 ± 0.2

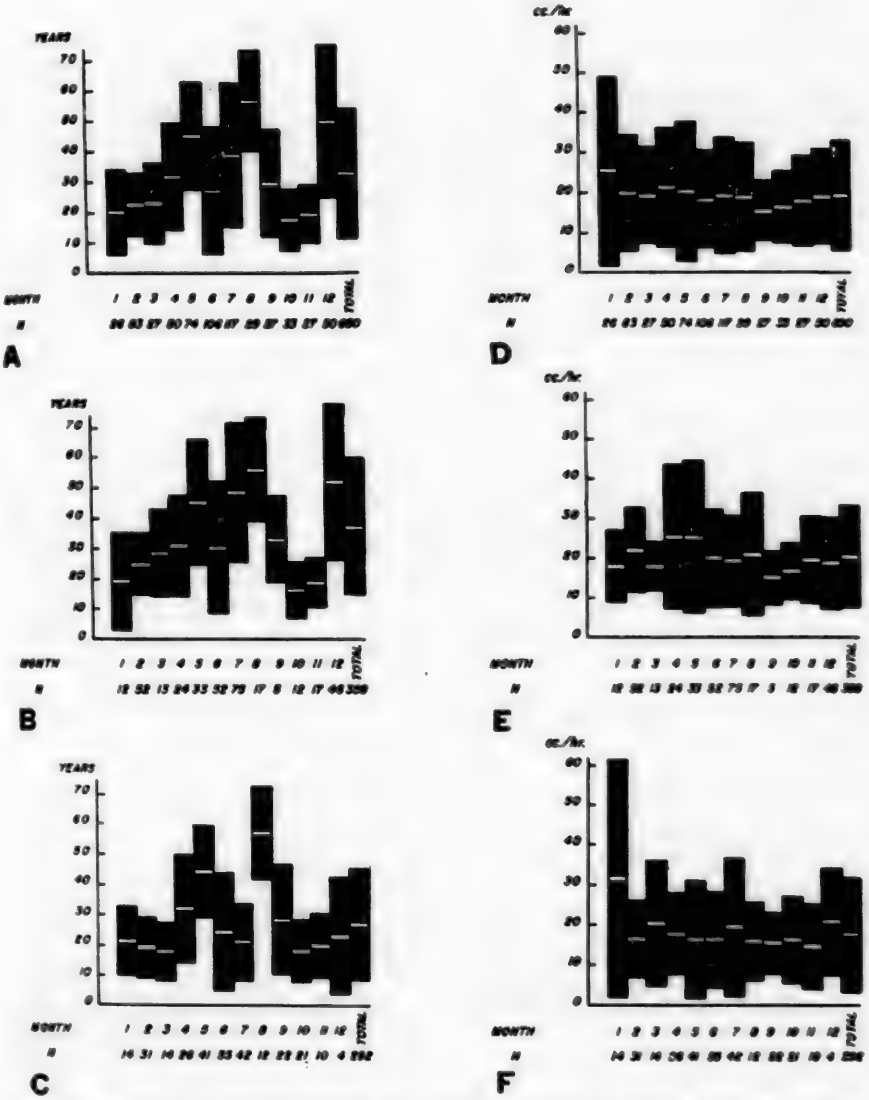

Fig. 1. Mean ±1 standard deviation of age (A, B, and C) and rate of flow (D, E, and F) arranged according to the month in which sample was analyzed. (A) and (D) total group, (B) and (E) males, (C) and (F) females.

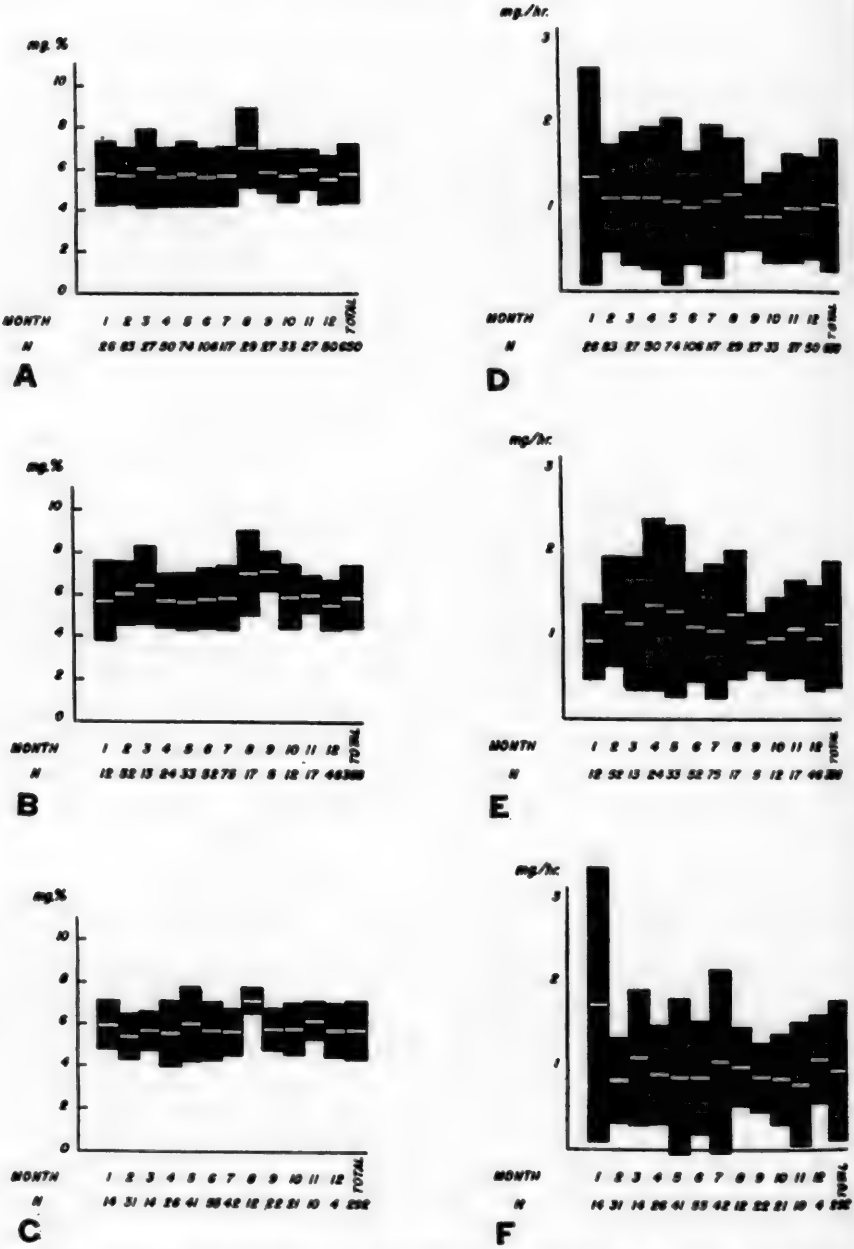

Fig. 2. Mean ±1 standard deviation of total calcium mg.% (A, B, and C) and mg./hr. (D, E, and F) values arranged according to the month in which sample was analyzed. (A) and (D) total group, (B) and (E) males, (C) and (F) females.

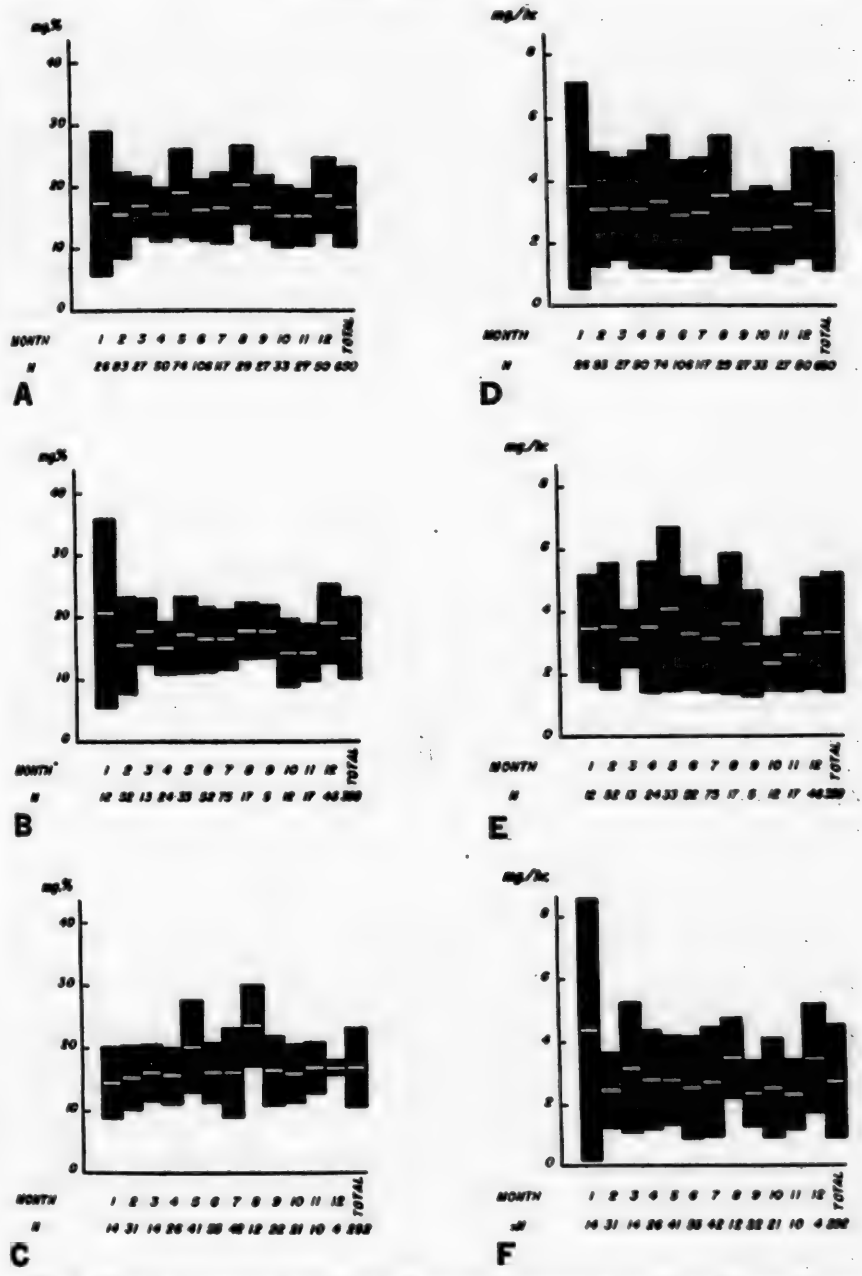

Fig. 3. Mean ±1 standard deviation of inorganic phosphorus mg.% (A, B, and C) and mg./hr. (D, E, and F) values arranged according to the month in which sample was analyzed. (A) and (D) total group, (B) and (E) males, (C) and (F) females.

may be noted in August with the highest average, which is the result of the high average age in this group. The *mg./hr.* values of calcium (*figs. 2D, E* and *F*) are also fairly constant throughout the year. The standard deviations indicate a wide range of observations in each month.

The *inorganic phosphorus mg.%* content (*figs. 3A, B,* and *C*) as well as the *mg./hr.* values (*figs. 3D, E* and *F*) show remarkably little variation.

TABLE II

Individuals with rates of flow of less than 0.5 cc./hr.

MONTH OF ANALYSIS	AGE, YEARS	SEX
July	72	♂
July	74	♂
July	75	♂
July	77	♂
July	85	♂
July	94	♂
Sept	58	♀
Dec	70	♂
Dec	70	♂
Dec	76	♂
Dec	77	♂

SUMMARY AND CONCLUSIONS

Single salivary analyses of 650 different individuals accumulated over a period of 3 years were sorted and combined according to the month of examination and studied for possible seasonal effects. The results *did not disclose any seasonal influence on rate of flow, calcium or phosphorus content of resting saliva.* This is in contrast to the previous report of Brawley and Sedwick in which a seasonal variation in calcium content was observed among different individuals.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

FOUNDED IN NEW YORK, DECEMBER 10, 1920

PROCEEDINGS OF THE TWENTY-FIFTH ANNIVERSARY CELEBRATION[1]

Held Under the Auspices of the New York Section, Men's Faculty Club, Columbia University, New York December 14th, 1945

COMPILED BY FRANCES KRASNOW

Guggenheim Dental Clinic, New York, N. Y.

There was a quiet, hopeful atmosphere—thoughts turning to the many members still overseas. Perhaps this made the cocktail hour so brief, though ample time was allowed. And so, reminiscing and discussion continued in the dining hall.

Taking part were H. K. Addleston, Mrs. Addleston, E. D. Becker, F. E. Beube, Mrs. Beube, G. Bevelander, T. Blum, R. H. Brodsky, Mrs. Brodsky, D. Brown, E. O. Butcher, L. Cahn, N. W. Chilton, G. W. Clapp, J. A. Cutitta, M. Diamond, J. R. Dunning, B. R. East, F. Ellis, L. Fox, W. J. Gies, J. F. Hall, S. Hemley, Mrs. Hemley, I. Hirschfeld, H. Holliday, T. Kaletsky, M. Karshan, M. Klatsky, Mrs. Klatsky, F. Krasnow, W. Lefkowitz, W. C. MacTavish, J. O. McCall, A. H. Merritt, S. C. Miller, Mrs. Miller, I. Neuwirth, Mrs. Neuwirth, C. R. Oman, B. B. Palmer, W. Rosenbaum, R. M. Rankow, Mrs. Rankow, S. N. Rosenstein, Mrs. Rosenstein, I. Salman, J. A. Salzmann, Mrs. Salzmann, J. Schroff, B. Seidler, Mrs. Seidler, H. H. Shapiro, S. Sorrin, G. Stein, L. R. Stowe, D. Tanchester, B. Tannenbaum, Mrs. Tannenbaum, B. O. A. Thomas, L. M. Waugh, Mrs. Waugh, L. Wolf, E. V. Zegarelli, Mrs. Zegarelli, D. E. Ziskin, Mrs. Ziskin.

Some fervently recalled annual dinners of the association's earlier days. The younger members felt secure in its continuity of purpose—advancement in dental science. Columbia University, Guggenheim Dental Clinic, Montefiore Hospital, New York University, The Institute of Clinical and Oral Pathology and the profession generally were represented. Dr. J. Frank Hall brought greetings from Indianapolis.

The dinner committee did not doubt that all of the group would agree to try out some newer knowledge in nutrition when they chose a menu which comprised a wholesome fruit-vegetable excess to provide an optimal balance in positive-negative ion replenishement. The service was not hurried but was completed nicely in deference to the speakers.

Dr. Ziskin, president of the New York Section, opened the after-dinner program promptly. Sincere thanks with spontaneous applauds were accorded Dr. Neuwirth for his wholehearted hard work in planning the occasion; but above this, however, for procuring the services of so public spirited an attorney as Emanuel D. Becker. It was very fitting to have Mr. Becker a guest at this twenty-fifth anniversary celebration for he won the case of the New York Section

[1] Received for publication May 10, 1946. The cost of publication of these proceedings, is paid, in part by The New York Section.

in proving that any presentation before its scientific sessions may be questioned by the association and can be withheld from inclusion in its published proceedings if so determined. Keen appreciation was shown.

Warm good wishes were extended the organization by Dean Brown of New York University and Dean East of Columbia University. Smiles of congratulations were then directed to the charter members seated on the dais as Dr. Ziskin named each of them—Theodore Blum, George W. Clapp, William J. Gies, Arthur H. Merritt, Bissel B. Palmer and Leuman M. Waugh.

Dr. Merritt spoke briefly, yet forcibly on making this year the twenty-fifth year of the founding of the International Association for Dental Research, the year for the completion of the $50,000 Endowment Fund to insure the continued publication of the JOURNAL OF DENTAL RESEARCH, financially unhampered. With only $10,000 more to go, the goal is within view. To reach it in fact depends upon the earnest effort of all of us to see the task through by giving a little more and getting our dentist and physician friends here and from other sections to do their bit. The JOURNAL OF DENTAL RESEARCH is a most important organ in Research.

Dr. Ziskin then called upon Dr. Gies who presented an outline of the history of the International Association for Dental Research. For use at this meeting, he received from Secretary Hatton an official statement (November 21, 1945) of current statistical data, which included the following: There are now 22 sections in the U. S. (organized from 1920 to 1944), 3 in Canada (organized from 1921 to 1930), and 1 each in Vienna (1929), London (1931), Prague (1932), Budapest (1934), Chengtu (1934), Johannesburg (1934), and Palestine (1940)—a total of 32 sections (1920–44). The total membership is 635, of whom 530 are resident in North America—506 in the U. S.; 24 in Canada. There are 105 members in Europe, Asia and Africa, of whom 33 are not members of sections. The two largest sections are those in New York and Chicago with 67 and 66 members respectively. The average membership of the 32 sections is 16. Of the total membership, 115 are not members of sections, most of them residing where sections have not yet been organized. The Association's bi-monthly JOURNAL OF DENTAL RESEARCH is about to begin its 24th annual volume.

In tracing the origin of the International Association for Dental Research, Dr. Gies recalled that in the spring of 1909 he, as head of the Department of Biological Chemistry in the Medical School of Columbia University, was invited, by the committee of a local dental society, to conduct for them a study of the relations between the condition of the teeth and the composition of the saliva. He said that at that time he—like all his colleagues in the Columbia University Medical School and in medical schools generally—had very little scientific knowledge about the teeth and had not been interested in any phase of dental research. But he was greatly impressed by the committee's earnest manifestation of the *spirit of research*, and felt impelled to accept their invitation despite his deficiency in knowledge and experience in the dental field.

The dental research thus began in 1909, and the associated studies of the related biological literature, soon led Dr. Gies to realize that the general inattention,

in science and in medicine, to the biology of the teeth and to this division of health service, had been detrimental to the public welfare. His continuing dental researches with the cooperation of an increasing number of associates—conducted primarily as exploratory inquiries in different aspects of dental physiology and pathology—created the further belief that the health relationships of the teeth would not receive adequate fundamental attention until there could be developed conditions that would stimulate and support dental research by many competent and interested workers under favorable educational, scientific and professional influences. By 1916 these convictions were formulated into a comprehensive plan to endeavor to bring about cumulative improvement in the standards of dental education, of dental journalism and of dental research. He then initiated the correlated movements that directly resulted successively in the establishment of the first university dental school in New York City—in Columbia University (1916) and, after postponements caused by World War I, of the JOURNAL OF DENTAL RESEARCH (1919), the International Association for Dental Research (1920), and the study of dental education in the U. S. and Canada under the auspices of the Carnegie Foundation (1921–26).

In conclusion, Dr. Gies read several portions of his early outlines of the history of the International Association for Dental Research, as published in the issues of the JOURNAL OF DENTAL RESEARCH for March 1926 and February 1931, including this quotation from page 59 of the latter (from the proceedings of the tenth anniversary meeting):

"Our Association exemplifies an impersonal ideal. Through the cooperation of accredited investigators in the sciences upon which dentistry rests and which it uses, the Association promotes the discovery of truth and the dissemination of new knowledge to the end that, irrespective of beliefs, opinions, policies, or traditions, oral health-service in all its aspects may be given its greatest possible usefulness for the whole of humanity. With this history behind us and with this ideal before us we may proudly and hopefully look forward to the evolution of endless decades in the growth and increasing public value of our Association".

Finally, all turned to John R. Dunning for the discourse of the evening on Atomic Energy. Despite the vastness of the subject and the inexpediency of disclosing certain details, the presentation was most interesting. Said Dr. Dunning: It is erroneous to herald the bombs over Hiroshima and Nagasaki as beginning a new age. Actually, the event was only a tiny spectacular result of work that has gone on for well over fifty years. In good part, the findings will eventually be of more service to all of us than to physicists.

He traced the development of our knowledge from the earliest idea of the atom as the unit in the structure of elements to the concept that it is a miniature solar system with central proton and outer revolving electrons. Then he explained the recognition of neutrons, the existence of isotopes, the search for how they differed, why physicists concentrated on inventing ways to speed up the movement of electrons and the use of the cyclotron to fire down into the central core thereby forcing combination not only of outer particles but also of inner ones. Close observations on the characteristics of uranium and the versatility of the

oscillograph in testing procedures of controlling neutron activity, finally led to the desired speed for optimal chain reactions. Further advances are now predictable. Concerted effort must be directed to harness this remarkable penetrating atomic power for easier and better living, for if used as a weapon the most ghastly devastation will be unloosed.

Hardly had Dr. Dunning completed, when he was bombarded with questions of scientific, political and social intent. Discussion continued unabated till a late hour when Dr. Ziskin called for adjournment. Thanks and applauds to Dr. Dunning brought the enjoyable silver jubilee to a close.